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THE INFLUENCE OF INSULIN ON THE PROTEIN METABOLISM OF NEPHRECTOMIZED DOGS[1]

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An examination of the prevailing views concerning the influence of insulin on protein metabolism suggests that this hormone exerts a paradoxical action.

In experimental or clinical diabetes mellitus, insulin produces a profound decrease in urinary nitrogen excretion which is concomitant with an increase in carbohydrate retention. The converse is revealed when insulin is withdrawn from the diabetic organism. On the other hand, in the normal organism, dog or man, the administration of insulin is reported to result in either no effect or in an increased urinary nitrogen excretion (1, 2). That the latter is the more frequent effect is suggested by the studies of Milhorat and Chambers (3) who observed that insulin induces an increase in nitrogen excretion when administered to dogs during the early stages of inanition when the stores of protein presumably are still adequate. During the latter stages of inanition, they could demonstrate no significant effect of insulin administration on nitrogen output. The studies of Luck and his co-workers (4) are in accord with the view that insulin accelerates the rate of amino acid catabolism, thus accounting in part for the decrease in the amino acid N content of the blood which occurs after the administration of insulin.

In view of these apparently contradictory effects of insulin, we considered it of value to reinvestigate this problem by methods which perhaps would indicate the actual influence of this hormone on the catabolism and anabolism of protein in the muscles and liver.

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METHODS. The classical procedure employed in studies on the protein metabolism of the intact organism consists of determining the rate of nitrogen elimination in the urine. In a previous study (5) we employed the rate of non-protein-nitrogen accumulation in the blood after bilateral nephrectomy as an index of the rate of protein catabolism and found this procedure to be as adequate as the more usual method. Furthermore, for reasons which will be discussed subsequently, we considered it of some importance to obviate any specific effect that the kidney may exert on protein catabolism, a condition which is possible only in the bilaterally nephrectomized animal. Hence, this procedure was employed throughout this study.

Two sets of animals were studied: "normal" nephrectomized dogs and completely eviscerated dogs. The abdominal eviscerations were performed by a modification of the method of hepatectomy described by Markowitz, Yater and Burrows, and the blood sugar was maintained at a normal level by a constant intravenous injection of glucose.

After a preliminary fast of 96 hours, the animal was anesthetized with sodium amytal and maintained so throughout the experimental period. Immediately after completion of the bilateral nephrectomy an arterial blood sample was drawn from an exposed femoral artery for the determination of non-protein-nitrogen by the micro-Kjeldahl method, sugar by the Somogyi modification of the Shaffer-Hartman method, amino-acid-nitrogen by the Danielson modification of the Folin method, and total solids by drying a weighed quantity of blood of constant weight. Subsequent arterial samples were drawn at two to four hourly intervals for various periods of time.

The experiments were divided into the following groups:

1. Eight dogs, nephrectomized and samples of blood drawn at intervals.

2. Nine dogs, nephrectomized and 0.1 unit of insulin per kilogram of body weight injected intravenously and at hourly intervals thereafter.

3. Ten dogs, completely eviscerated and the blood sugar maintained by the continuous intravenous injection of 0.14 gram of glucose per kilogram of body weight per hour.

4. Eight dogs, completely eviscerated and the blood sugar maintained by the continuous intravenous injection of 0.28 gram of glucose per kilogram of body weight per hour. One-tenth unit of insulin per kilogram of body weight was injected intravenously at the outset of the experiment and at hourly intervals thereafter.

5.[2] Seven dogs, completely eviscerated and the blood sugar maintained as in group 3. In addition, 600 mgm. of glycine (in 20 per cent solution) per kilogram of carcass weight were injected intravenously. Thirty

[2] Preliminary report made in the Proc. Soc. Exper. Biol. and Med. **37**: 223, 1937.

minutes were allowed for diffusion of the injected glycine before the first blood sample was drawn.

6.[2] Seven dogs, prepared as in group 5, but receiving a continuous injection of 0.28 gram of glucose per kilogram of body weight per hour, and in addition an intravenous injection of two units of insulin per kilogram of body weight 15 minutes after the administration of glycine and 0.1 unit of insulin per kilo of body weight at hourly intervals thereafter.

TABLE 1

The influence of insulin on blood non-protein and amino acid nitrogen of nephrectomized and eviscerated dogs

EXPERIMENTAL GROUP	NUMBER OF ANIMALS	BLOOD SUGAR, MGM. PER CENT					BLOOD N.P.N., MGM. PER CENT					BLOOD AMINO ACID NITROGEN, MGM. PER CENT				
		0	4 hrs.		8 hrs.		0	4 hrs.		8 hrs.		0	4 hrs.		8 hrs.	
		Actual	Actual	Change	Actual	Change	Actual	Actual	Change	Actual	Change	Actual	Actual	Change	Actual	Change
I. Control nephrectomized	8	64	63	−1.0	61	−3.0	29.3	40.1	+10.8	52.1	+22.8	6.8	6.6	−0.2	6.5	−0.3
II. Insulin, nephrectomized	9	85	49	−36	32	−53	31.1	37.1	+6.0	46.4	+15.3	7.5	6.0	−1.5	6.2	−1.3
III. Control, eviscerated	10		Maintained				31.4	38.0	+6.6	46.9	+15.5	7.9	13.6	+5.7	19.1	+11.2
IV. Insulin, eviscerated	8		Maintained				30.7	33.5	+2.8	35.6	+4.9	7.8	6.9	−0.9	8.4	+0.6

TABLE 2

The influence of insulin on the removal of exogenous amino acids from the blood of eviscerated-nephrectomized dogs

EXPERIMENTAL GROUP	NUMBER OF ANIMALS	BLOOD NON-PROTEIN-NITROGEN					BLOOD AMINO ACID NITROGEN				
		0	2 hrs.		4 hrs.		0	2 hrs.		4 hrs.	
		Actual	Actual	Change	Actual	Change	Actual	Actual	Change	Actual	Change
Glycine treated, eviscerated	7	58.5	57	−1.5	61.8	+3.3	32.8	30.6	−2.2	30.8	−2.0
Glycine plus insulin treated, eviscerated	7	67.8	53.5	−14.3	49.8	−18.0	36.4	23.5	−12.9	18.6	−17.8

RESULTS. The data obtained in the above experiments are summarized in tables 1 and 2. Those from the nephrectomized "normal" dogs (group I) are in accord with the figures of a previous set of experiments (5), indicating again the validity of our procedure for comparing the rates of protein metabolism under different conditions. It is of interest to

indicate that despite the degree of operative manipulation associated with the bilateral nephrectomy, the blood amino-acid-nitrogen and the blood sugar show insignificant fluctuations from the initial level.

The administration of insulin to the nephrectomized dog (group 2) results in a definite decrease in the blood sugar and in the rate of non-protein-nitrogen accumulation. Thus, while the non-protein-nitrogen in the blood of the control group rises at the rate of 2.8 mgm. per cent per hour, that of the insulin treated group rises at the rate of 1.9 mgm. per cent per hour. In accord with the observations of Luck and his co-workers is the decrease in the level of the blood amino-acid-nitrogen content. This decrease apparently is maintained, for it is observed throughout the experimental period.

In order to determine whether or not the influence of insulin is exerted on the muscles or upon the liver, we employed the eviscerated animal. Such animals may be regarded essentially to be muscle preparations. The data obtained from these animals (group 3) suggest that the liver, and perhaps other abdominal viscera, exert a significant augmentatory influence on the total protein metabolism of the organism. Thus, the rate of non-protein-nitrogen accumulation in the blood of the eviscerated group is 15.3 mgm. per cent for eight hours, as compared to 22.8 mgm. per cent for the same period of time for nephrectomized animals.

Since the liver and kidneys are the only tissues in which appreciable degrees of deaminization can occur (6, 7), the removal of these organs should result in a cessation of this process, and the rise in non-protein-nitrogen in the blood should primarily be due to an increase of amino-acid-nitrogen. That such is the case is indicated by the fact that of a total accumulation of 15.5 mgm. non-protein-nitrogen per 100 cc. of blood in 8 hours, 11.2 mgm. are due to the amino-acid-nitrogen fraction. Furthermore, since in the eviscerated animal the muscle is the main, if not the only site from which these amino acids can originate, it is probable that the rate of amino-acid-nitrogen accumulation in the blood of such a dog is an index of the balance between the hydrolysis and synthesis of protein in the muscles.

In figure 1 the data have been analyzed to reveal the influence of insulin on the various fractions of the blood non-protein-nitrogen. Since the major portion of the non-protein-nitrogen, other than the amino acids, is made up of urea, the non-protein-nitrogen minus amino-acid-nitrogen difference may be used as a rough measure of the blood urea nitrogen. On that basis, figure 1 suggests that the administration of insulin to nephrectomized dogs results in the removal of amino acids from blood and in a decrease in the amount of urea that is being formed; the latter being in accord with the observations of Bach and Holmes (8) on liver slices. In the eviscerated group, insulin administration results in a cessation of the

excretion of amino acids from the muscles. From these analyses it is suggested that insulin diminishes the rate of nitrogen catabolism by decreasing the production of amino acids in the muscles, and by inhibiting the deaminization of these acids in the liver.

In order to determine whether the decrease in the rate of amino acid accumulation in the blood of the insulin treated eviscerated animals is due to an actual decrease in protein hydrolysis or to an increase in amino acid retention in the muscles, we studied the influence of insulin administration on the utilization of administered amino acids.

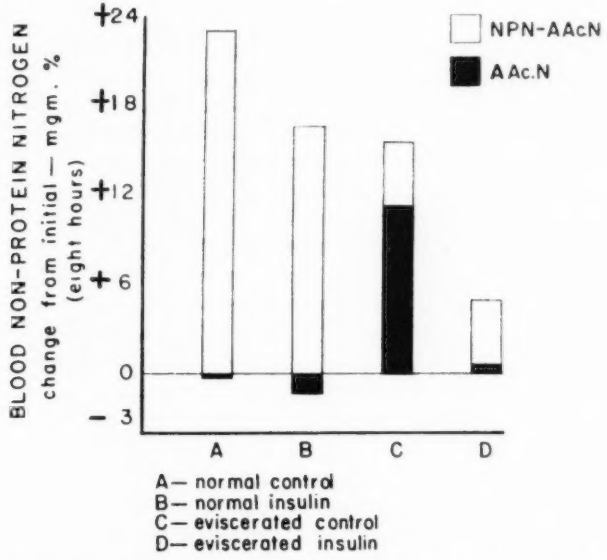

Fig. 1. Illustrating the influence of insulin on the various fractions of the non-protein-nitrogen accumulation in the blood of nephrectomized normal and eviscerated dogs.

Table 2 reveals that the intravenous administration of glycine results in a rise of the amino-acid-nitrogen level of the arterial blood to 32.8 mgm. per cent (group 5). After two hours, a negligible decrease in the amino acid nitrogen content of the blood is found. In the subsequent two hour period no further change is observed. The fluctuations in the total non-protein-nitrogen of the blood also are negligible throughout the period studied. Hence, it is obvious that deaminization does not occur to any significant degree in the absence of the liver and kidneys.

The effect of insulin administration in stimulating the removal of exogenous amino acids from the blood is very striking. Since the changes

in both amino-acid-nitrogen and non-protein-nitrogen are identical, it is probable that the decrease in blood amino acid nitrogen is not due to a breakdown of the molecules, but is due to the removal of these substances from the blood by the muscles.

Discussion. It is apparent from the preceding that insulin can exert a nitrogen-sparing effect in non-diabetic animals, and that this probably is due to a decrease in amino acid liberation by the muscles as well as a decrease in the deaminization of the amino acids by the liver. The fact that insulin induces a removal of exogenous amino acids from the blood suggests the probability that it stimulates the synthesis rather than inhibits the hydrolysis of muscle protein.

Our observations on nephrectomized dogs are not in agreement with the conclusions of many investigators who found that insulin administration either produces an increase in nitrogen excretion, or has no influence whatsoever. Neither are they in agreement with the data obtained in another investigation in which we studied the influence of insulin on the nitrogen excretion of a human male maintained on a constant diet for three months. In that instance, we observed no difference in the nitrogen excretion when the subject was treated with as much as 60 units insulin per day for many weeks, as compared to similar periods of time without insulin. However, in accord with our animal studies is the observation that the non-protein-nitrogen accumulation in the blood of patients suffering from chronic glomerulo-nephritis can be definitely decreased by the administration of insulin and high carbohydrate diets.

In view of the fact that in the present experiments we are dealing with nephrectomized animals, it is possible that the difference between our conclusions and those of others is due to some direct or indirect effect of insulin on the kidney which in turn influences the excretion of nitrogen. The experience of Jakobson is in accord with this possibility. He found that insulin, though inducing an increase in the excretion of nitrogen, does not affect sulfur excretion and hence concluded that insulin does not cause an increase in endogenous protein catabolism.

Since our data reveal that insulin can produce a sparing of nitrogen even in non-diabetic animals, it is possible that the paradoxical actions discussed at the outset are not indicative of variable changes in endogenous protein metabolism but are dependent upon the relationship between the effect of insulin on the liver and muscles (protein-sparing) and that upon the kidneys.

SUMMARY

The administration of insulin results in *a*, a decrease in the rate of the protein catabolism of the nephrectomized dog; *b*, a decrease in the rate of amino-acid excretion from the muscles of the eviscerated dog, and *c*,

an increase in the utilization of exogenous amino acids by the muscles of the eviscerated dog.

The data suggest that insulin exerts its nitrogen-sparing action by *a*, decreasing the rate of oxidative deaminization in the liver, and *b*, increasing the rate of amino acid utilization by the muscles for protein synthesis.

We wish to acknowledge gratefully the generosity of the Eli Lilly Company in providing us with generous supplies of sodium amytal and insulin.

REFERENCES

(1) Sokhey, S. S. and F. N. Allan. Biochem. J. **18:** 1170, 1924.
(2) Jakobson, B. M. and H. Reinwein. Arch. f. exper. Path. u. Pharmakol. **170:** 84, 1933.
(3) Milhorat, A. T. and W. H. Chambers. J. Biol. Chem. **127:** 595, 1928.
(4) Luck, J. M., G. Morrison and L. F. Wilbur. J. Biol. Chem. **77:** 151, 1928.
(5) Mirsky, I. A., J. D. Heiman and S. Swadesh. This Journal **120:** 681, 1937.
(6) Bollman, J. L., F. C. Mann and T. B. Magath. This Journal **69:** 371, 1924.
(7) Krebs, H. A. Biochem. J. **29:** 1620, 1935.
(8) Bach, S. J. and E. G. Holmes. Biochem. J. **31:** 89, 1937.

HIGH OXYGEN EFFECTS ON ISOLATED STRIATED MUSCLE[1,2]

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When an animal is exposed to oxygen at high barometric pressure, there occur reactions which, since the early work of Paul Bert (1878), have been known as oxygen poisoning. Of late years evidence has accumulated (Gesell, 1923; Bean, 1929, 1931; Campbell, 1929; Hill, 1933; Shaw, Behnke, and Messer, 1934) which quite definitely indicates that such poisoning is causally related to an accumulation of carbon dioxide and acidity in the tissues as a result of a failure in normal reduction of hemoglobin. This failure is due to the fact that at oxygen pressures exceeding three atmospheres sufficient oxygen for tissue needs may be supplied from the plasma without reducing the oxyhemoglobin. The question of whether high oxygen might not have some other and more direct effects contributory to acute oxygen poisoning, however, has not been satisfactorily answered. The study herein reported was undertaken with the hope of acquiring further experimental evidence bearing on this question.

APPARATUS AND PROCEDURE. Isolated frog muscle, muscle nerve, and non-circulated reflex preparations were used. These were mounted in a small compression chamber equipped to record muscle contractions photographically. The chamber temperature was held constant and the compression atmosphere—pure oxygen—was maintained saturated with water vapour by bubbling it through a glass bead humidifier. As an added precaution against drying, large area pans of water were placed within the chamber. Electrical stimulation was applied from a thyratron and induction coil. The stimulating electrodes employed were pin and non-polarizable silver-silver chloride-Ringer solution types. Blocks of single shock stimuli (each block made up of 10 single shocks at 3 second intervals) were applied at about 15 minute intervals. Identical stimulation was applied in similar manner to the control preparation (the mate of the test preparation, where possible) mounted in a moist chamber containing oxygen saturated with water and maintained at atmospheric pressure.

[1] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[2] Preliminary report: Proceedings, American Physiological Society, This Journal **123**: 11, 1938.

Test and control experiments were carried out simultaneously. Additional controls were run also in air at increased barometric pressure in the compression chamber.

RESULTS. *Muscle stimulation.* In these experiments the sartorius muscle with its insertion at the tibia was isolated and suspended in the compression chamber by a bone clamp. Stimulating electrodes were fixed on the nerve free end of the muscle and contractions were recorded photographically by an isotonic muscle lever.

The initial effect of high oxygen was a slight increase in the height of contraction, followed by a progressive decrease. Under prolonged exposure this decrease continued to complete cessation of response. Sharp

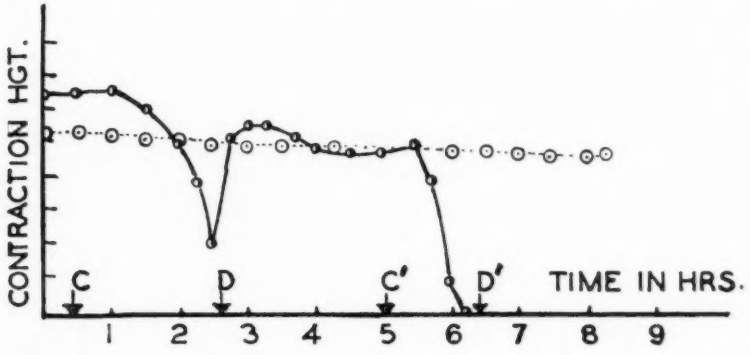

Fig. 1. Direct muscle (sartorius) stimulation. Continuous line curve represents contraction height of test preparation. At C the oxygen pressure was raised to 5 atmospheres. At D the pressure was lowered to atmospheric. C^1 and D^1 mark a second compression and decompression respectively. Contraction height of the control preparation maintained in oxygen at 1 atmosphere pressure is represented by the broken line.

declines in the contraction height appeared as early as one and one-half hours after compression to five atmospheres, but usually the onset of these oxygen effects was more delayed and occasionally did not appear until after nine hours of exposure. Decompression to atmospheric pressure was attended in some experiments by a partial but distinct recovery, the extent of which was inversely related to the duration of exposure to high pressure. In no experiment, however, was there complete recovery. These effects are illustrated in figure 1. Here the progressively decreasing height of contraction induced by the compression, C, continued until decompression, D, which was followed by a partial recovery. Exposure of this same muscle to high oxygen a second time resulted in a much earlier onset of, and a sharper gradient in, the decrease of response. Decompression, D^1,

from this second exposure failed to give any recovery. In contrast to these effects the contractions of the control preparation had decreased but slightly even two hours after the test preparation had ceased to respond.

Muscle nerve preparation. In experiments on nerve muscle preparations the sartorius muscle together with its motor nerve was isolated and mounted in the pressure chamber. Electrical stimulation was applied to the nerve through non-polarizable silver-silver chloride-wick electrodes.

Exposure to oxygen at slightly more than 5 atmospheres' pressure gave effects essentially similar to those recorded in the direct muscle stimulation experiments. Results typical of these experiments are shown in figure 2. Contractions of the test preparation and those of the control were recorded simultaneously. At 12:55 both preparations were stimulated in oxygen at one atmosphere. The pressure was then raised, *C*, in the chamber to slightly more than 5 atmospheres.

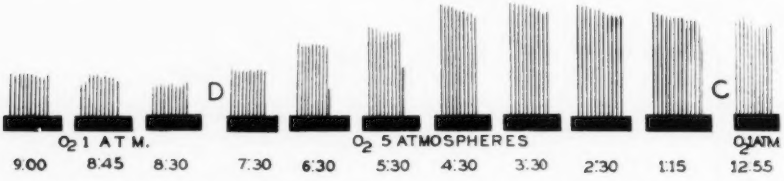

Fig. 2. Sartorius muscle contractions elicited by motor nerve stimulation. At *C* the oxygen pressure was raised to five atmospheres. At *D* the oxygen pressure was lowered to atmospheric pressure. Time of day is indicated below each block of contractions.

The initial effect of high oxygen was an increase in height of contraction. This increase was maintained for about 4 hours after which time the contraction height decreased progressively for 3 hours. Lowering of the oxygen pressure, *D*, to atmospheric slowed the rate of decrease in height of contraction and brought about a partial but definite recovery. A second compression one hour later (not shown in figure) again turned the height of contraction downward. This second compression was not attended by an increase in height of contraction; on the contrary, a decrease became immediately evident and the progress of deterioration was more rapid than on the initial compression, so much more so that whereas the initial exposure decreased contraction to 50 per cent of its pre-compression height only after six hours, the second exposure brought about a similar decrease within less than one hour. Moreover, decompression after the second exposure failed to bring about any evidence of recovery. Apparently the deleterious action of high oxygen in this isolated tissue has a cumulative effect from which there is only partial recovery. The control

preparation contractions, on the other hand, had decreased only slightly even after ten hours. It is interesting to note also (fig. 2) that in addition to the alteration in height of contraction, the treppe phenomenon which was quite apparent in both the control and test preparation records at the beginning, disappeared from the test preparation contractions at the time when the high oxygen had induced a decrease in the height of contraction. Some recovery of the treppe phenomenon appeared after the first decompression *D* (8:45), but was rapidly dispelled by the second compression. In the control preparation however the treppe persisted throughout the whole experiment.

Reflex preparations. Attempts were made to study the effect of high oxygen on isolated reflex systems. For this purpose the semitendinous muscle, spinal column with cord, afferent and efferent nerves were isolated and the whole mounted in the pressure chamber. It was found that the rapid deterioration of such preparations precluded the possibility of obtaining any conclusive evidence regarding the effect of high oxygen on these isolated arcs. The high oxygen did not appreciably prolong the irritability of the preparations. The records suggest that high oxygen may extend the latent period and diminish the after discharge of the arc.

Discussion. High oxygen may exert deleterious effects on living organisms without the involvement of the hemoglobin dysfunction mentioned above. For example, Novy and Soule (1925) found that certain bacteria were killed by exposure to high oxygen pressure. Moreover, in animals breathing gas mixtures containing oxygen in quantities greater than 70 per cent over extended periods, there occur irritative reactions in pulmonary tissue, even though the barometric pressure is maintained at one atmosphere. Smith (1899) found that oxygen at high barometric pressure had some acute irritative influence which gave rise to a pneumonic condition of the lungs. The disturbance in carbon dioxide transport and the increasing acidity of the tissues under such experimental conditions, however, make it difficult to obtain conclusive evidence of a direct acute oxygen toxicity on circulated animal tissues.

The results of our experiments on isolated frog muscle indicate there is some "direct" but delayed toxic action on tissues. The delay may have been due in part to a slowed diffusion rate in the non-circulated muscle, but this cannot be the whole explanation since the high diffusion gradient obtaining under the increased barometric pressure should more than offset the condition of non-circulation. The delay in appearance of any direct adverse effect would more likely be due to a low order of oxygen toxicity.

The nerve fibre is no more susceptible to this "direct" toxic action than is the muscle. This is brought out by a comparison of the effects of oxygen

in the experiments on muscle stimulation with those recorded during motor nerve stimulation. Similarly the neuromyal junction is apparently no more susceptible than is muscle itself.

Just how this "direct" toxic influence of high oxygen acts on muscle and possibly to a less degree on nerve fibre and the neuromyal junction is not known. In earlier experiments (Bean and Haldi, 1932) it was suggested that oxygen poisoning involved injury or poisoning of enzymes concerned in tissue oxidation. Various investigators have found oxidations in circulated animals to be diminished as a result of exposure to high oxygen (Bert, 1878; Smith, 1899; Hill and Macleod, 1903; Bean, 1931). It must be admitted, however, that in circulated preparations, an increasing tissue acidity consequent upon the involvement of hemoglobin dysfunction may also be contributory to the lowered oxidations and this perhaps through the influence of acidity on enzymatic processes. But there is also evidence that oxidations in isolated tissue may be diminished by high oxygen acting on enzymatic processes. Meyer (1927) found that brain tissue which has been exposed to oxygen under pressure, is less efficient in causing the oxidation of guaiacum than brain tissue which has been exposed to atmospheric air for an equal period. He concluded that "oxygen at a pressure of four atmospheres appears to exert a definite retarding influence on the metabolic activity of brain tissue." Librecht and Massart (1937) in their oxygen studies found that high oxygen poisons the dehydrogenase and thereby decreases oxygen consumption. Lehman (1935) found that oxygen in his experiments acted toxically on enzyme solutions only in the presence of succinic acid.

The cumulative effect of high oxygen in successive exposures and the incomplete reversibility of effects as recorded in our experiments is in line with the findings of Almeida (1934) that an animal apparently free from permanent ill effects of one large dose of oxygen, is sensitized and succumbs much more quickly to a second administration. Moreover, Lehman (1935) in experiments with enzyme solutions has found that oxygen at high tensions irreversibly injures the oxidation system. These findings help to explain the incompletely reversible effects of oxygen poisoning in some prolonged exposures of circulated animals.

Since the "direct" toxicity of high oxygen on peripheral tissue as discussed here has been with few exceptions so uniformly slow in manifesting itself, whereas in the circulated animal signs of toxicity become evident shortly after compression, it appears that the failure in reduction of hemoglobin at the tissues is a much earlier and more important factor in oxygen poisoning. It is not denied, however, that the "direct" toxicity of high oxygen may play an important rôle centrally even though these experiments indicate the nerve fibre and neuromyal junction are no more and perhaps less susceptible to "direct" toxicity than is striated muscle.

Lehman (1935) reports that the enzyme solutions studied in April and May were not so markedly affected by high oxygen as were those studied in November to March. He attributes the difference to a yearly change in the oxygen handling systems of the cell wherein hormones and vitamins are concerned. This may be an explanation of why the "direct" toxic effect of oxygen was less pronounced in our experiments carried out in April than those of November and December.

SUMMARY AND CONCLUSIONS

Isolated frog muscle, muscle nerve, and reflex preparations were exposed in a compression moist chamber to oxygen at five atmospheres' pressure. Single shock electrical stimulation from a thyratron stimulator was applied to these preparations by pin or non-polarizable electrodes. Muscular contractions were recorded photographically. Control experiments were performed with the mate of the test preparation in oxygen at atmospheric pressure and in air at increased pressure.

The initial effect of high oxygen was an increase in the height of contractions elicited either by direct electrical stimulation of the muscle or of the motor nerve. Subsequently there occurred a progressive decrease in height of contraction, which appeared as early as one and one-half hours after compression, but usually did not appear until after six hours of exposure.

Exposure to high oxygen caused a disappearance of the treppe phenomenon, concurrent with the decrease in contraction height.

High oxygen apparently exerted a toxic influence on isolated noncirculated tissue. This deleterious effect was not completely reversible and there was a cumulative effect of successive exposures.

So far as can be judged from the height of contraction, the nerve fibre or the neuromyal junction was no more and perhaps was less profoundly affected by high oxygen than was the muscle itself.

The "direct" toxic action of high oxygen is most likely due to a poisoning of enzymes involved in oxidative processes.

Unless central structures are much more susceptible than peripheral nerve fibre neuromyal junction, or striated muscle, to direct toxic action of high oxygen, the accumulated carbon dioxide and acidity resulting from inadequate reduction of hemoglobin at the tissues during exposure of circulated animals to high oxygen, remains the more significant etiological factor in the early signs and later events of oxygen poisoning.

REFERENCES

ALMEIDA, A. O. DE. Compt. rend. Soc. de Biol. **116**: 1225, 1934.

BEAN, J. W. Proc. Soc. Exper. Biol. and Med. **26**: 832, 1929.

J. Physiol. **72**: 27, 1931.

BEAN, J. W. AND J. HALDI. This Journal **102**: 439, 1932.

BERT, P. La Pression Barometrique. Paris, 1878.
CAMPBELL, J. A. J. Physiol. 68 P. vii, 1930.
GESELL, W. This Journal **66**: 5, 1923.
HILL, L. Quart. J. Exper. Physiol. **23**: 49, 1933.
HILL, L. AND J. J. R. MACLEOD. J. Hyg. **3**: 27, 1903.
LEHMAN, J. Skandinav. Arch. f. Physiol. **72**: 78, 1935.
LIBBRECHT, W. AND L. MASSART. Compt. rend. Soc. de Biol. **124**: 299, 1937.
MEYER, A. L. This Journal **82**: 370, 1927.
NOVY, F. G. AND M. H. SOULE. J. Infect. Dis. **36**: 92, 1925.
SMITH, J. L. J. Physiol. **24**: 20, 1899.
SHAW, A. L., A. R. BEHNKE AND A. C. MESSER. This Journal **108**: 652, 1934.

THE ORAL ASSAY OF ADRENAL CORTICAL HORMONE

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We wish to report the method of oral assay of the life maintaining potency of the adrenal cortical hormone used successfully by us for over a year. In addition we wish to point out certain factors—seemingly inherent to any oral assay method—which must be taken into account in establishing hormone potency, and an Oral Rat Unit.

The literature reveals a variety of methods for the parenteral assay of the active principle of the adrenal cortex. These assays are performed on different species and give results which are, admittedly, of considerable variation. The units of potency in most general use are: the dog unit, suggested by Swingle, Harrop, and their co-workers (5, 7), and the several "rat units" proposed by Kutz (6), Grollman and Firor (4), Cartland and Kuizenga (1), and others. The "rat units" are established in an essentially similar manner, but differ in the degree of hormone replacement required as a measure of potency, and give "units" which are not of equal size.

That a preparation designed for oral administration should be assayed orally is self-evident. It would seem advisable, also, that the physiological action of any oral unit, which might be generally accepted, should be reasonably comparable in physiological potency to the parenteral units in general use. Grollman (3) uses the same measure of potency in his oral as in his parenteral assays. Recently, D'Amour and Funk (2) have proposed a less rigorous oral assay, suggesting that an oral rat unit be the amount of hormone necessary per rat per day to give a 10 per cent weight gain over a period of 10 days, the animals being operated at about 30 days of age.

METHOD AND RESULTS. The assay to be described in detail, and the one upon which the data in figure 1 and table 1 are based, represents the result of considerable experimentation with the various factors which enter into the determination of the power of an orally administered cortical hormone preparation to maintain life and normal physiological conditions in bilaterally adrenalectomized young rats.

The hormone preparation used is, in every instance, a charcoal adsorbate of the adrenal cortex, prepared in commercial quantities in this laboratory

by a modification of the method described by Grollman and his collaborators (4).[1]

The rats used in this work are a pure Long-Evans strain of hooded rats and are bred in this laboratory. The animal room, and operating room, are kept at a uniform temperature (72–78°F.), and humidity throughout the year. All the animals in the colony receive a stock, adequate diet prepared in a uniform manner by the caretaker.

The details of the assay follow:

The young are weaned at 21–23 days, at which time they weigh about 30 to 35 grams, and are allowed to remain together in the breeding cages for 2 to 3 days to become accustomed to being without the mother. At this time, when they weigh about 35 to 40 grams, they are put in the individual cages in which the assay is to be carried out, and are allowed to become acclimated to these conditions before the operation. A daily record of the animals' weight gain is kept, and the animals are bilaterally adrenalectomized by a one stage operation on the day when their weight most nearly approaches 50 grams. With few exceptions all the animals are operated between the weights of 48 to 52 grams, and at this time their age is, on the average, 30 to 32 days. This somewhat involved and careful technique in the pre-operative preparation of the assay animals gives a group of rats which are as nearly uniform in all respects as seems possible to secure. Following the operation the animals are fed, in non-scatter food cups, the normal stock diet to which has been added varying amounts of the charcoal adsorbate to be assayed. Daily weighings of the food intake and growth are made, and at the end of 14 days (day 15 of the assay period, counting the day of operation as day 1) the hormone preparation is removed and the animals given the same stock diet without the hormone. Daily records are continued until the animals die of cortical insufficiency, or, as in a few instances, live until autopsy,[2] at which time gross examination almost invariably shows the presence of adrenal cortical tissue at, or near, the site of the operation. It is probable that these cortical rests represent regenerated remnants, rather than accessory tissue. When the assay is completed the weight gain of the assay animals is compared with that of normal, unoperated controls. From the accumulated data, it is possible to calculate the amount of food eaten, and the amount of hormone received by each rat per day.

At the time of writing over 500 rats have been used in the development of the assay method, and the hormone preparation.

A composite, graphic record of the assay results obtained by this method is shown in figure 1.

While this chart and its associated description is self-explanatory, some further discussion of the results seems advisable at this point.

Following the removal of the hormone on day 15, the curves are continued until the death of many of the rats makes the figure of no statistical significance as representing the average weight of the group. We have selected day 24 as the time to

[1] The charcoal adsorbate of adrenal cortex used in this study is available commercially as Cortisorbate Tablets.

[2] Two adrenalectomized controls did not die. Upon autopsy adrenal cortical tissue was found.

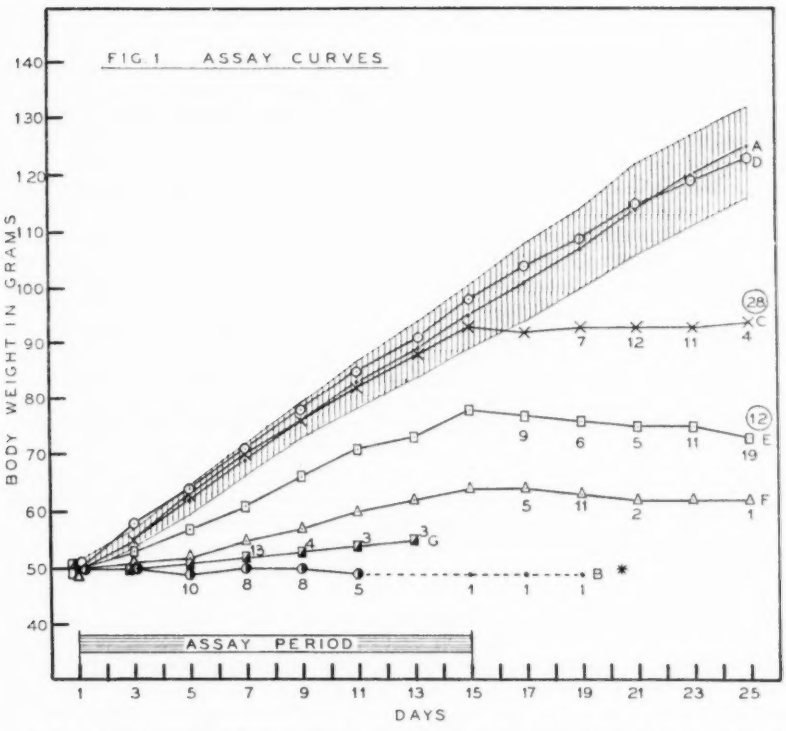

Fig. 1. Arabic numerals on curves *B*, *C*, *E*, *F* and *G* indicate the number of animals dead on the days shown.

Curve A: Average, normal growth curve. Lined area shows distribution of growth, or "normal growth zone."

Curve B: Growth curve of operated, untreated controls.

Curve C: Growth curve of operated animals which received sufficient charcoal adsorbate of adrenal cortex to enable them to show normal growth. Average, daily hormone intake—0.24 gram.

Curve D: Growth curve of adrenalectomized animals which did not die following the assay, but came to autopsy. Upon gross examination all showed remnants of adrenal tissue remaining.

Curve E: Growth curve of adrenalectomized animals receiving a lesser amount of hormone than those of curve C. Did not show normal growth. Average daily hormone intake—0.16 gram.

Curve F: Growth curve of adrenalectomized animals which ate but an average daily intake of 0.11 gram of hormone. Showed very little gain, although they were able to live through the assay period.

Curve G: Growth curve of adrenalectomized animals which did not receive sufficient hormone to give any effective replacement of the extirpated glands. These animals did not survive the assay. Average daily hormone intake—approximately 0.05 gram.

N.B. Numbers in the circles at the ends of curves C and E indicate the number of assay rats still alive on day 25. All subsequently died. Average survival time of all assay animals after removal of the hormone—8.8 days.

stop the plotting of the curves although the collection of the data continues until the death or autopsy of all animals. In some instances, as in curves B, F, and G, the curves are stopped even earlier, as too few rats remained alive to give any meaning to further average figures. As is to be expected, some of the rats receiving hormone for the two weeks assay period live longer, following the removal of the adsorbate, than the operated controls which receive no hormone therapy at all. The average life span is, however, essentially the same; 8.5 days for the bilaterally adrenalectomized control rats receiving no hormone, and 8.8 days for the survival period of assay rats after removal of the hormone. The assay period, without doubt, enables the animals to recover from any effect of shock, and slight trauma incidental to the operation, although with proper technique these effects can be minimized. Five rats after assay lived longer than 21 days without hormone, and 36 survived longer than 10 and less than 21 days. As might be expected, the longer survival times were in those groups of rats receiving the higher amount of charcoal adsorbate. With the exception of two animals which were autopsied and shown to have cortical tissue remaining, all of the operated control rats died within 18 days, and only 6 lived longer than 12 days. Of the entire group of over 500 rats used in the course of these experiments, but 4 per cent of the adrenalectomized animals, both untreated controls and assay rats off hormone, failed to die in the absence of any therapy, and of this small group, only 3 failed to show small bits of cortical tissue remaining in the body. It is probable that serial sections cut through the region of the operation would have disclosed cortical remnants in these three also. The average weights on curves C, E, and F do not show an appreciable decrease following the removal of the hormone from the diet, but rather a cessation of growth. This is in agreement with reports in the literature that rats, contrary to the condition encountered in dogs and cats, do not necessarily lose weight as a factor in the development of symptoms of adrenal cortical insufficiency. Curve A is the normal growth curve for the animals of this strain growing in this laboratory, and the cross-hatched area represents the normal "growth zone" for these same rats. An assay animal, in order to be considered as showing "satisfactory growth," must have a growth curve falling within this zone, or a minimum average growth of 2.5 grams per day. Curve D represents the growth of rats, presumably adrenalectomized, but living indefinitely, and which on autopsy showed "accessory" tissue. This group of animals has no bearing upon the assay results but is included for the sake of completeness.

As previously mentioned, figure 1 is a composite summary of the data representing the entire group of over 500 rats, which have been used to assay 10 different batches of charcoal adsorbate by this method. Inasmuch as these several hormone batches showed varying degrees of potency, it is impossible to determine a unit from these cumulative data. An Oral Rat Unit as defined and used in this laboratory is: *The amount of hormone material per day which will maintain life and normal growth for a period of 14 days in 80 per cent of a group of not less than 10 bilaterally adrenalectomized rats, operated when approximately 50 grams in weight and which die following the removal of the hormone.* In table 1 are given the results of a single assay from which such a unit can be determined.

It is obviously impossible to find any large number of rats which, of their own volition, will eat exactly the same amount of material per day

during the course of fourteen days. This fact prevents the absolute regulation of oral hormone intake such as can be obtained by parenteral administration. Consequently, it appears impossible to define an oral

TABLE 1

Assay data of batches M-6 and M-7

ANIMAL	AVERAGE WEIGHT GAIN PER DAY	AVERAGE HORMONE INTAKE PER DAY	SURVIVAL PERIOD AFTER ASSAY
48	4.2	0.18	23
76	4.5	0.17	10
1	2.1	0.15	6
17	3.3	0.27	7
56	2.5	0.15	16
11	3.2	0.20	9
57	1.6	0.15	8
6	3.8	0.21	10
37	3.4	0.20	5
41	2.8	0.20	5
5	3.3	0.20	8
68	2.8	0.18	8
9	3.1	0.18	17
49	2.8	0.17	15
2	2.7	0.17	16
12	2.9	0.15	5
33	3.6	0.15	10
64	2.4	0.10	15
4	0.5	0 05	2
15	2.6	0.13	9
39	2.1	0.08	2
38	1.2	0.07	4
14	2.3	0.10	22
28	2.4	0.08	10
31	1.7	0.06	13
79	2.1	0.11	12

Five rats eating less than an average of 0.10 gram of adsorbate per day, died during the assay and are not included in the above table. Four animals did not die following removal of the hormone and, upon autopsy, adrenal tissue was found by gross examination. Animals in the upper group ate 0.15 gram, or more, of the adsorbate. All but two or 87 per cent of this group gained satisfactorily. From this *One Oral Rat Unit* is equivalent to 0.15 gram of charcoal adsorbate. The animals in the lower group ate less than an average of 0.15 gram of material per day, and but one showed satisfactory growth.

unit in terms of the "least amount" which will produce a given result, inasmuch as individual variations in food intake cannot be prevented. Therefore, considerable care must be exercised in determining the unit in

any individual assay. For example, as illustrated by table 1, a definite "break" occurs at 0.15 gram of adsorbate per day. All those receiving that amount or more gained satisfactorily within the limits of the definition of the unit, while only one of those eating less than 0.15 gram showed satisfactory growth. Therefore, in this particular assay, 0.15 gram of charcoal adsorbate is taken as representing one Oral Rat Unit. We have found that such a division always occurs among those animals which survive the assay period, providing a sufficient number are taken for assay, and that at least ten animals on each percentage level of hormone in the food seem adequate.

In addition to the method described in detail above, other variations of the procedure have been tried. Extension of the assay period from 14 to 21 days showed no significant difference in results from the shorter assay, and was abandoned as unecessarily prolonging the test period.

In the early stages of the investigation, the use of a salt-free assay diet was tried. The animals appeared not to relish the diet, and it was difficult to get the rats to eat sufficient of this salt-free diet to keep even normal unoperated rats in a well nourished condition. Since in our hands the normal stock diet gave satisfactory results, there seemed to be no reason to persist in the use of this special food. In those instances where a salt-free diet might be definitely indicated, considerable improvement in the general course of the experiment can be obtained by tiding the animal over the first few days after the operation by permitting it to drink 0.9 per cent NaCl rather than pure water. Under these conditions, the actual assay period would begin from the day upon which the salt water was withdrawn.

Discussion. There are two fundamental ways in which an oral assay may be performed, or oral therapy carried out. The first is to feed the animal a definite amount of the material being studied by stomach tube, capsule, or some other convenient method. This procedure seems quite feasible with larger animals but very impractical with small rats. The second method consists of mixing the material to be studied with the diet and allowing the animal to have free access to the food. Using the rat, this method has one advantage; the eating habits of the rat, differing from those of the larger laboratory animals, result in the animal receiving an almost continuous supply of hormone material, since the rat eats small amounts of food at frequent intervals. There are certain difficulties, however, which seem to be inherent in any method in which hormone intake is dependent upon the voluntary consumption of food by the assay animal. For example: consider the case of an animal being maintained on barely sufficient hormone intake to give a normal weight gain. For some reason, change in humidity, fright, noise, etc., the rat may eat less than the customary amount for this particular day, and thereby lower

appreciably the hormone intake for the day. With an animal on a barely sufficient diet, this one day's decrease in hormone may result in a loss of appetite which is reflected in a further decrease in food intake the following day. Thus a "vicious circle" of appetite decrease-hormone decrease is set up which may result in the death of the animal from cortical insufficiency, even though the diet being eaten contains enough hormone to maintain normal physiological conditions with normal food intake. The converse situation arises in which an animal eating a small amount of food and receiving barely enough hormone to maintain life, suddenly increases its daily food and hormone intake to a level where normal weight gain takes place. Thus, for the latter part of the assay, the rat is gaining satisfactorily, but due to the initial lag, the total weight gain for the whole assay period is below normal. Another difficulty, which can be minimized, however, by the use of a sufficiently large number of rats, lies in the fact that the weight gain of the animals is actually dependent upon two factors: first, the amount of food eaten, and second, the amount of hormone received, and these two factors themselves are interdependent. Thus, as is obvious, it is very difficult to determine the exact critical level which would divide a barely adequate hormone intake from one which is definitely insufficient. This critical division would only be possible if all the animals on the assay ate the same amount of food per day, regardless of the percentage of hormone in the diet.

The method of assay, and the Oral Rat Unit as described above, seem to be as satisfactory for general oral use as can be devised on the basis of our experience. Contrary to the results reported by Grollman (3), it seems impossible, in our experience, because of individual variations and the difficulties inherent in any oral assay, to set a standard of 100 per cent satisfactory response to a definite minimum hormone intake. We feel that in demanding an 80 per cent response from one oral rat unit, we can obtain a fairer evaluation of the true life maintaining potency of the hormone material, and at the same time establish a definitely rigorous unit. That this 20 per cent leeway works out satisfactorily in practice seems evident from an examination of the data and discussion of table 1.

It seems evident that no direct comparison of oral and injectable units can be made. Material intended for oral administration must be separately assayed by oral methods. Any of the recognized units may be adapted to such preparations if the material is assayed orally and the units should be stated as "oral" dog units, "oral" cat units, or "oral" rat units, as the case may be.

As far as we are aware, but two methods, primarily designed for oral assay, have been described in the literature. That proposed by Grollman (3) is one, and that of D'Amour and Funk (2) the other. Our assay as described above closely approximates that of Grollman, but we have been

unable to attain that almost mechanical perfection which he reports, and we feel that the allowance we have made in the definition of an Oral Rat Unit makes it of much more general application. The assay method proposed by D'Amour and Funk is much less rigorous than the one we here outline, and while their assay may be quite satisfactory for qualitative determination of the presence of the life maintaining principle of the adrenal cortex, their proposed unit seems inadequate to indicate properly the actual physiological potency of a hormone preparation. We therefore propose this Oral Rat Unit for general acceptance in the assay of the potency of the life maintaining hormone of the adrenal cortex in any such preparation intended for oral administration.

The authors wish to thank Dr. Robert Gaunt and Mr. Potts of Washington Square College, New York University, for their assistance in carrying out the assays with the salt-free diet, and Mr. E. A. Jenkins, of this laboratory for technical assistance in the recent assays.

SUMMARY

A method for the oral assay of the potency of the life maintaining principle of the adrenal cortex has been described.

An Oral Rat Unit defined as "The amount of hormone material per day which will maintain life and normal growth for a period of 14 days in 80 per cent of a group of not less than 10 bilaterally adrenalectomized rats, operated when approximately 50 grams in weight and which die following the removal of the hormone" has been proposed.

Difficulties which appear unavoidable in oral assays in which the hormone intake is conditioned by voluntary food consumption of the experimental animals have been discussed.

REFERENCES

(1) Cartland, G. F. and M. H. Kuizenga. This Journal **117:** 678, 1936.
(2) D'Amour, F. E. and D. Funk. J. Pharmacol. **62:** 307, 1938.
(3) Grollman, A. The adrenals. Williams & Wilkins, Baltimore, 1936.
(4) Grollman, A., W. M. Firor and E. Grollman. J. Biol. Chem. **109:** 189, 1935.
(5) Harrop, G. A., J. J. Pfiffner, A. Weinstein and W. W. Swingle. Proc. Soc. Exper. Biol. Med. **29:** 449, 1932.
(6) Kutz, R. L. Proc. Soc. Exper. Biol. Med. **29:** 91, 1931.
(7) Pfiffner, J. J., W. W. Swingle and H. M. Vars. J. Biol. Chem. **104:** 701, 1934.

THE SUPERNORMAL PERIOD IN THE MAMMALIAN VENTRICLE[1]

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Exploration of the excitability during recovery from previous response of various nerve and muscle preparations has revealed a period of heightened excitability following the relatively refractory period. Since the threshold at this time is found to be lower than subsequently or at rest, this phase has been designated as the supernormal period. This period of enhanced excitability may exist normally or may be produced by such agents as fatigue, acid, or veratrine. In the frog ventricle a supernormal period was found by Adrian (1921) when the fluid bathing the tissue was acidified, and Wastl (1922) detected supernormality in fatigued preparations. Eccles and Hoff (1934) noticed occasional supernormality in the pacemaker of decerebrate cats, while Lewis and Master (1924) and later Rothberger and Goldenberg (1936) presented evidence for its existence in the conduction system.

The object of this study has been to determine 1, whether a supernormal period exists in the mammalian ventricle; 2, what its position is in respect to the ventricular electrocardiogram, and 3, what factors may influence its occurrence.

METHODS. Twenty-three cats were anesthetized with sodium amytal (12), dial (1) or nembutal (1), or were decerebrated by the trephine method during deep ether narcosis (9). A mid-sternal skin incision permitted detachment of the left pectoral muscles from the sternum, and the heart was then exposed by removal of a segment of overlying rib. While artificial respiration was maintained, the pericardium was opened, and its edges sewn to the margins of the thoracic opening so that the anterior surface of the heart was exposed and the pleural cavities closed. Artificial respiration was discontinued and the animal resumed spontaneous respiration.

After a delay of half an hour to permit reëstablishment of equilibrium following possible overventilation from the artificial respiration, a silver electrode was hooked into the epicardium and a second electrode inserted into the anus. The thoracic opening was then surrounded by cotton pledgets moistened with saline and covered by a sheet of cellophane. A lamp placed close to the opening kept the heart warm, and permitted observation, while the cellophane prevented drying. Rhythmic

[1] This work was aided by a grant from the Fluid Research Funds of the Yale University School of Medicine.

induction shocks of varying intensity were delivered at an interval of a second from a thyratron stimulator, while electrocardiograms were taken from lead II. Thirty to fifty shocks permitted exploration of the excitatory cycle at any one intensity, and several such series made possible the construction of the complete time-intensity curve.

The supernormal period was considered to be that period in diastole in which weaker shocks were effective in evoking a response and were again ineffective during the remainder of diastole.

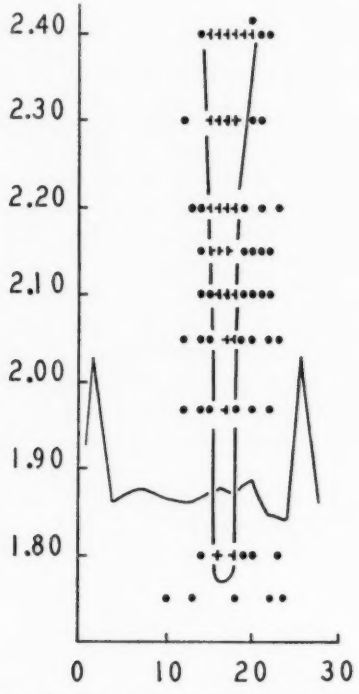

Fig. 1. March 23, 1938; 3.5 kgm. cat. Sodium amytal intraperitoneally 70 mgm. per kgm. Recovery curve of point on anterior surface of heart near apex. Ordinates, strength of stimuli in rheostat readings. (Reading minus 1.0, times 90 = peak voltage of induction shock. Abscissae intervals in hundredths of a second after Q of the previous normal complex. Crosses represent effective stimuli, and dots represent ineffective stimuli.) The electrocardiogram from lead II is outlined on the same time scale, and shows a U wave coinciding with the supernormal period.

RESULTS. A. The fourteen animals anesthetized with barbiturates invariably showed a supernormal period subsequent to the relative refractory period, lasting 0.04 to 0.10 sec. In all cases this period fell either on the terminal portion of the T wave, or, if a U wave was present, as it was in over half the records, it fell on the U (fig. 2). These relations are shown

in figure 1, which indicates the extreme degree to which the supernormal period was present, and shows the part of the electrical cycle during which it occurred. The least pronounced supernormal period showed a threshold only 10 volts below normal.

B. Seven of the nine decerebrate animals showed no supernormal period while in two a very shallow period of supernormality was found.

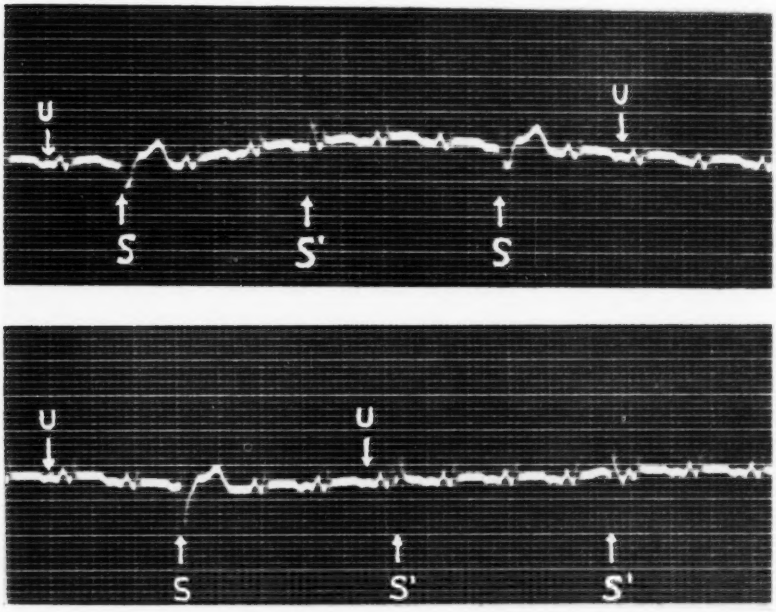

Fig. 2. Electrocardiograms from two different experiments showing the supernormal period falling on a U wave. Above, two effective stimuli marked by S, and a slightly later ineffective stimulus at S^1. Below, a stimulus at the apex of U is effective while one at the end of T is ineffective.

Discussion. The mammalian ventricle is shown by these experiments to be no exception to the general rule that irritable tissues may in the proper circumstances exhibit a period of supernormal excitability following the relatively refractory period. As in other tissues the supernormal period of the cat's ventricle is not a constant phenomenon, but may be entirely absent, or may vary widely in degree when it is present. It happened that the two types of preparation employed gave almost diametrically opposite results; in barbiturate anesthesia the supernormal period was invariably present, while in the decerebrate animal it was nearly always absent. The known effect of the barbiturates to produce an acidosis may

account for its presence in amytalized animals, although the influence of the drug on the autonomic nervous system (Meek and Seevers, 1934) or upon the heart directly must be considered. The presence of a shallow supernormal period in only two of the nine decerebrate preparations corresponds to its infrequent occurrence in the pacemaker after decerebration (Eccles and Hoff, 1934). Several known factors which might account for this are the increased activity of the sympathetic nervous system found in decerebrate animals, the augmented metabolism reported by Dusser de Barenne (1924), and the possible effects of decerebration on the respiratory center to produce overventilation.

The constant association of the supernormal period in nerve with the negative after-potential implies strongly the existence of a similar relationship in the ventricle. That this does in fact occur in the heart is supported by the following evidence: 1. In every experiment showing supernormality this period fell during continuing electrical activity as shown by the electrocardiogram. In records in which there was no U wave the supernormal period coincided with the terminal portion of T, and when a U wave was present, the supernormal period fell during this phase. 2. In each experiment the cycle of excitability was determined at a different point on the anterior surface of the ventricle, without in any way altering the above relationship. It appears improbable that the point of exploration could in every case have been electrically inactive while the rest of the heart was active as shown by the electrocardiogram. 3. It is known that the entire ventricle is activated within a very short time, probably less than 10 msec., whereas the extent of the supernormal period is much greater than this. Since it is probable that the cessation of electrical activity will occur in all parts of the ventricle with a simultaneity equal to its onset, the region explored must have been electrically active during the supernormal period.

The supernormal period has been shown to occur either during the final portion of the T wave or during the U wave when this wave was present. The U wave must therefore be considered to be a part of the ventricular complex, indicating the position of the supernormal period.

CONCLUSIONS

1. A supernormal period is invariably found in the ventricle of cats under barbiturate anesthesia.

2. The supernormal period is usually not found in the ventricle of decerebrate cats.

3. Supernormality is associated with the end of the T wave, or with the U wave.

4. The U wave is part of the ventricular electrical complex.

REFERENCES

ADRIAN, E. D. J. Physiol. **55:** 193, 1921.
WASTL, H. Ztschr. f. Biol. **75:** 289, 1922.
ECCLES, J. C. AND H. E. HOFF. Proc. Roy. Soc. **115B:** 307, 1934.
LEWIS, T. AND A. M. MASTER. Heart **11:** 371, 1924.
GOLDENBERG, M. AND C. J. ROTHBERGER. Pflüger's Arch. **237:** 295, 1936.
MEEK, W. J. AND M. H. SEEVERS. J. Pharmacol. Exper. Therap. **51:** 287, 1934.
DUSSER DE BARENNE, J. G. J. Physiol. **59:** 17, 1924.

CHANGES IN FAT, CARBOHYDRATE AND PROTEIN APPETITE IN VITAMIN B DEFICIENCY

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The results of previous experiments have amply demonstrated the ability of adrenalectomized and parathyroidectomized rats to select those substances (sodium chloride, calcium lactate) necessary for the maintenance of life (Richter, 1936; Richter and Eckert, 1937). More recently, it has been shown that normal rats, offered a variety of purified foodstuffs, make dietary selections which result in normal growth and reproduction (Richter, Holt and Barelare, 1938). Also, pregnant and lactating rats choose their diet in accordance with their needs (Richter and Barelare, 1938).

The present paper deals with the application of this self-selection method to the study of vitamin B deficiency, in which there are known to be derangements of metabolism. Biochemical studies and non-selective feeding experiments indicate disturbed carbohydrate metabolism and more efficient fat utilization in animals deprived of vitamin B (Thompson and Johnson, 1935; Evans and Lepkovsky, 1929). On the other hand, little is known concerning protein utilization during this deficiency. It becomes of interest, therefore, to learn whether these alterations in fat and carbohydrate metabolism are reflected in the animal's appetite, and whether there are any alterations in protein appetite which might suggest abnormal protein utilization.

METHODS. Rats at an average age of 52 days were placed in individual cages, consisting of a small living compartment (with one food receptacle and one graduated inverted water bottle) and a revolving drum with a cyclometer attached. Daily records of activity, food and water intake, and vaginal smears were kept; the animals were weighed weekly.

Base lines of normal growth and activity were obtained on the standard McCollum diet for approximately 20 days before starting the self-selection diet. Then the small living compartment was replaced by a large cage containing 3 food cups for sucrose, casein (autoclaved and purified), and dried baker's yeast, and 5 graduated inverted bottles for olive oil, cod

liver oil, sodium chloride, 3 per cent, calcium lactate, 2.4 per cent, and water. The diet was made deficient in vitamin B by omitting the yeast.

Since yeast contains not only vitamin B_1 (thiamine chloride), but also riboflavin, nicotinic acid, B_6, and at least one other active substance, the W factor, the vitamin B deficiency of these preliminary experiments is obviously a deficiency involving at least 5 substances.

Nine animals were used in the present study. Yeast was omitted from the diet until the presence of vitamin B deficiency was established, approximately 30 days later. The following criteria were used: 1 *Weight loss.* This was a marked and rapid loss. Some of the animals suffered a reduction of $\frac{1}{3}$ of their body weight within 30 days. 2. *Decreased running activity.* In most instances there was a temporary slight increase in activity during the first 10 days on the deficient diet, but the activity fell to a constantly low level soon after. 3. *Constant dioestrous phase of the vaginal epithelium.* The cornified cells which normally appear every 4 to 5 days disappeared completely after 2 to 3 weeks on the deficient diet. 4. *Facial dermatosis.* This was characterized by a scaling and reddening of the skin of the face, especially marked around the eyes and about the snout. 5. *Polyneuritis.* Progressive paralysis of the muscles, particularly the muscles of the hind legs, was observed. At the height of the deficiency, a number of the rats dragged their hind legs about in the manner characteristic of the polyneuritic animal. Figure 1 is the chart of a typical animal in this group. It will be seen that the deficiency period is characterized by rapid loss of weight, decrease in activity and absence of cornified cells in the vaginal epithelium.

Results. Coincidentally with the development of the deficiency state, the rats showed marked changes in their food intake. Figure 2 is the record of a typical animal in this group, showing the changes in olive oil, sucrose, and casein intake during the deficiency period and after yeast was added. It will be seen that the protein (casein) and carbohydrate (sucrose) intake fell rapidly as the deficiency progressed, whereas the fat (olive oil) intake increased markedly. When access was given to yeast, the rat displayed a craving for it and consumed large quantities. Shortly after, the protein and carbohydrate intake increased and the fat intake fell to its pre-deficiency level. Simultaneously the signs of vitamin B deficiency disappeared (see fig. 1). Body weight increased rapidly. In fact, some of the animals doubled their weight after 30 days. Regular 4- to 5-day oestrous cycles were reëstablished after 15 to 25 days. There was an increase in running activity; most of the animals regained their original activity levels in 20 to 30 days.

Figure 3 summarizes the results for the entire group. This shows the average food and caloric intake for the periods before deficiency, at the height of the deficiency, and after the deficiency was corrected with yeast.

It will be seen that the average total fat, carbohydrate, and protein intake, measured in grams, fell from 8.8 grams to 3.3 grams (63 per cent decrease) during the deficiency period. After yeast was offered the total intake increased to 10.5 grams. In the same way the total caloric intake dropped from 42.2 to 24.4 (43 per cent decrease) during the deficiency period and increased to 48.3 after access to yeast was given. Before the deficiency was established, fat (olive oil) provided 23 per cent of the calories, carbohydrate (sucrose) 51 per cent, and protein (casein) 26 per cent. At the height of the deficiency, fat supplied 84 per cent, carbohydrate 11 per cent,

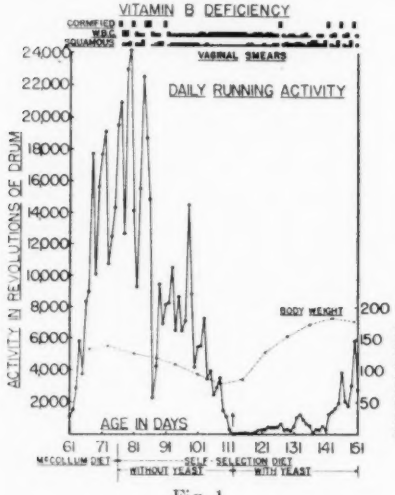

Fig. 1

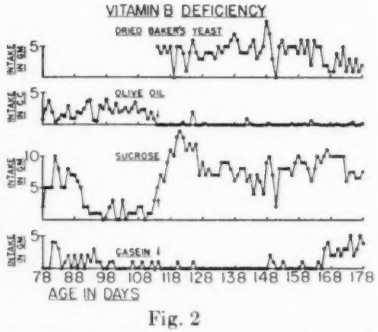

Fig. 2

Fig. 1. Chart of a typical animal showing the weight loss, dioestrous phase of the vaginal epithelium, and reduction in running activity during vitamin B deficiency. Correction of these signs after addition of yeast is indicated.

Fig. 2. Graphic representation of the changes in appetite for sucrose, olive oil, and casein before deficiency, at the height of the deficiency, and after correction of the deficiency by the addition of yeast. This is the daily intake record of a typical animal.

and protein 5 per cent. After recovery by yeast ingestion, fat provided 14 per cent, carbohydrate 56 per cent, and protein 30 per cent of the calories.

Table 1 shows the consistency of these appetite changes. Nine animals decreased their carbohydrate (sucrose) and protein (casein) intake, and 8 of the 9 animals increased their fat (olive oil) intake during the deficiency period. After the ingestion of yeast with subsequent recovery from the deficiency, 8 of the 9 rats ate more carbohydrate and 8 drank less olive oil. Only 5 animals increased their casein intake, whereas 4 showed no

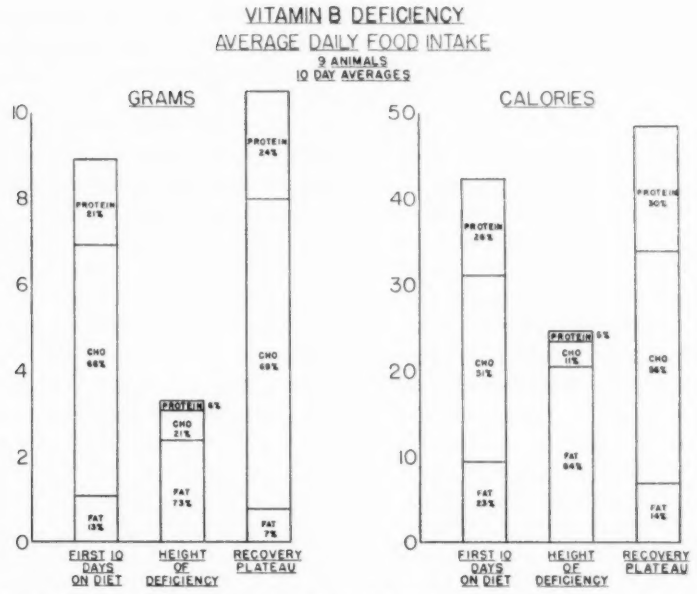

Fig. 3. Chart showing the changes in the proportions (calories and grams) of fat, carbohydrate, and protein ingested before deficiency, at the height of the deficiency, and after recovery with yeast.

TABLE 1

Table giving the number of animals showing changes in appetite for olive oil, sucrose, and casein during the deficiency and after recovery with yeast

	DEFICIENCY PERIOD	RECOVERY PERIOD
	Number of animals	
Sucrose:		
Decrease	9	
No effect		1
Increase		8
Olive oil:		
Decrease		8
No effect	1	1
Increase	8	
Casein:		
Decrease	9	
No effect		4
Increase		5

effect. This is undoubtedly due to the fact that the yeast contributed a great deal of protein. (This yeast contains 48 per cent protein.) It was noted that if the animals were allowed continuous access to the yeast, the yeast intake decreased somewhat after the vitamin craving was satisfied. With this decrease in yeast intake, the casein intake increased.

Most of the animals showed an increased calcium lactate appetite after recovery with yeast. There was a less definite increase in sodium chloride appetite and no appreciable change in cod liver oil intake.

Discussion. It is a well-known fact that vitamin B deficient animals rapidly lose weight. This weight loss has been shown to be due to the anorexia which accompanies the deficiency state. As shown above, rats on a vitamin B deficient self-selection diet also display anorexia and decrease their daily food intake by 63 per cent.

The present study demonstrates, however, that the anorexia is really a loss of appetite for carbohydrate and protein, whereas the fat appetite is actually increased. Apparently, the carbohydrate anorexia is a result of the disturbed carbohydrate metabolism during vitamin B privation. Kinnersley and Peters (1930) reported an increased level of lactic acid in B_1 deficient pigeons' brains. Others (Thompson and Johnson, 1935; Peters and Thompson, 1934; Johnson, 1936; Platt and Lu, 1936) have advanced evidence to show that there are increased amounts of pyruvate (bisulphite-binding substances) in the blood during vitamin B deficiency. On the other hand, vitamin B deficient animals display signs of deficiency much later when fed a diet with a high fat-low carbohydrate content (Salmon and Goodman, 1937). Indeed, Evans and Lepkovsky (1929) have stated that fat has a "sparing-action" on vitamin B.

Thus, just as glandular deficient animals exhibit an increased appetite for those substances which are necessary to life, and just as normal animals, and pregnant and lactating animals, show appetites for substances in proportion to their needs, so vitamin deficient animals seem to reflect their needs in their appetites. Vitamin B deficient rats have an aversion for carbohydrate, which they cannot utilize, and a craving for fat which appears to be beneficial. The avidity with which these deficient rats consumed yeast after it was made accessible should also be emphasized in this connection. Harris and co-workers (1933) found that vitamin B deficient rats, given a choice between a high vitamin B diet and a low, chose the former. In a previous communication, it was reported that vitamin B deficient rats display a remarkable craving for crystalline vitamin B_1, and also riboflavin (Richter, Holt and Barelare, 1937).

It appears, then, that the ability or inability of an animal to utilize a substance can be deduced from its appetite for that substance. The aversion for protein displayed by these rats suggests strongly, therefore, a derangement of protein utilization, although there are few biochemical

data at hand concerning protein metabolism under conditions of vitamin B privation. McClure, Voris and Forbes (1934) have stated that there is an increased digestibility of protein during vitamin B deficiency, but this does not necessarily indicate increased utilization. The disturbance is more likely one of intermediary metabolism as in the case of carbohydrate. Nasset, Pierce and Murlin (1931) have reported that yeast feeding in normal and pancreatectomized dogs results in a prompt decrease in the proportion of ingested nitrogen eliminated in the urine. Further investigation should lead to the discovery of a metabolic basis for the appetite change.

It is concluded that these appetite changes, viz., the craving for fat and yeast and the aversion toward protein and carbohydrate, are as characteristic signs of vitamin B deficiency as the anorexia, weight loss, activity decrease, bradycardia, and the altered state of the vaginal epithelium.

SUMMARY

1. Nine animals on an adequate self-selection diet of pure foodstuffs were rendered deficient in vitamin B by the omission of yeast from the diet.

2. These animals lost weight rapidly and became inactive. The vaginal epithelium was of the dioestrus type. All of the animals showed signs of polyneuritis and facial dermatosis.

3. At the height of the deficiency, the animals displayed a marked aversion for carbohydrate and protein and a craving for fat. The average daily protein intake decreased from 26 to 5 caloric per cent, carbohydrate intake decreased from 51 to 11 caloric per cent, and the fat intake increased from 23 to 84 caloric per cent.

4. The carbohydrate aversion and fat craving are explained in terms of the altered metabolism of these substances during vitamin B deficiency.

5. The aversion toward protein suggests a disturbance of protein utilization which has not yet been detected by biochemical methods.

6. Given access to yeast, the animals consumed it eagerly and thus corrected the deficiency.

7. These changes in appetite, the aversion toward carbohydrate and protein, and the craving for fat and yeast, further characterize the vitamin B deficient state.

REFERENCES

Evans, H. M. and S. Lepkovsky. J. Biol. Chem. **83:** 269, 1929.

Harris, L. J., J. Clay, F. J. Hargreaves and A. Ward. Proc. Royal Society B **113:** 161, 1933.

Johnson, R. E. Biochem. J. **30:** 31, 1936.

Kinnersley, H. W. and R. A. Peters. Biochem. J. **24:** 711, 1930.

McClure, F. J., L. Voris and E. B. Forbes. J. Nutrition **8:** 295, 1934.

NASSET, E. S., H. B. PIERCE AND J. R. MURLIN. J. Lab. and Clin. Med. **16:** 12, 1151, 1931.
PETERS, R. A. AND R. H. S. THOMPSON. Biochem. J. **28:** 916, 1934.
PLATT AND LU (cited by R. A. PETERS). Lancet **1:** 1161, 1936.
RICHTER, C. P. This Journal **115:** 155, 1936.
RICHTER, C. P. AND B. BARELARE, JR. Endocrinology **23:** 15, 1938.
RICHTER, C. P. AND J. F. ECKERT. Endocrinology **21:** 50, 1937.
RICHTER, C. P., L. E. HOLT, JR. AND B. BARELARE, JR. Science **86:** 2233, 354, 1937. This Journal **122:** 734, 1938.
SALMON, W. D. AND J. G. GOODMAN. J. Nutrition **13:** 477, 1937.
THOMPSON, R. H. S. AND R. E. JOHNSON. Biochem. J. **29:** 694, 1935.

ACTIVE CHANGES IN SIZE AND DISTENSIBILITY OF THE AORTA DURING ACUTE HYPERTENSION

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One of us (C. J. W.) recently reported (7) that the aortae of normal dogs, left *in situ* and perfused with constant pulse volumes immediately after death, expand sufficiently up to very high internal pressures, so that progressive increases in peripheral resistance consistently reduce the pulse pressure. These results were contrary to predictions based on volume-elasticity curves said to characterize excised rings or cylinders of aorta some time after death. They were contrary also to observations on living dogs that the pulse pressure increases as a result of intense reflex vaso-constriction. The latter are unquestionably due in part to the augmented systolic output which secondarily accompanies such vaso-constriction. The possibilities nevertheless exist that the vessels possess a different modulus of elasticity during life or that they may undergo changes in caliber; either or both of which might contribute to the augmentation of pulse pressure.

This communication analyzes experiments performed to test such possibilities. The investigation consisted in comparing systolic and diastolic diameters of the thoracic aorta with systolic and diastolic pressures at the root of the aorta, while internal pressures rose to hypertensive levels as a result of pressor reflexes and pressor hormones. We consider changes in the diameter of the aorta preferable to volume records because the former can be recorded free from longitudinal thrusts and do not necessitate disturbances of the aorta in its natural sheath. Thus, any nervous influences that may exist can continue to operate.

Procedure. All studies were made on dogs anesthetized with morphine and sodium barbital. Under mild artificial respiration the left thorax was opened and the thoracic aorta was exposed. A small aortagraph[2] was firmly stitched to its walls, taking care that the small levers were attached in the diameter of the vessel. The stem of the instrument was firmly

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[2] Either the instrument described on previous occasions as the segment myograph (6) or that called the jewelled-bearing myograph (4) formed a suitable aortagraph.

clamped so that accidental movements of the chest wall, elongation of vessel, etc., could not be recorded. The aortagraph was connected by lead tubing to a segment capsule, the sensitivity of which determined the recorded amplitude. Meticulous attention was paid to the maintenance of a leak-proof system. It may be noted that aortagrams so recorded differ from sphygmograms in that no significant counterpressure was applied to the aorta at any time. Calibration proved that the pressure created within the system never exceeded a few millimeters of H_2O and that the records are very nearly linear with separation of the lever arms; hence they give qualitative changes in the diameter of the aorta at any moment of the heart cycle.

Optical pressure curves were recorded as usual, by means of a calibrated Wiggers manometer, from the root of the aorta. This accounts for the slight delay in the rise of the aortagram and minor variations in contour of the systolic summits. Previous tests had shown however that the amplitude of the pressure variations undergoes no significant changes during this short distance of transmission; hence the convenience of recording pressures from the root of the aorta can be utilized.

Distensibility curves of the living dog's aorta. Since each experiment yielded long continuous photographic records, only selected segments can be published, to illustrate particular phases. Some of the results can be reported by use of distensibility curves of the living dog's aorta, plotted from records in which the pressure rose through induction of peripheral constriction. As illustrated in figure 4-D the diastolic and systolic pressures were determined in successive pressure pulses and used for abscissal values. The corresponding diastolic and systolic diameters, measured from an arbitrary base line as illustrated at *a* and *b*, figure 4, were employed as ordinate values for the respective pulses. Since with rising pressures, the diastolic pressures reach levels which exceeded systolic pressures at the start, overlapping points were obtained in the middle portions of such curves. Their good correspondence validates the method of studying diameter changes. This is illustrated for example by figure 1 in which the dots represent diastolic $\frac{p}{d}$ relations and the crosses systolic $\frac{p}{d}$ relations.

Figure 1 shows three distensibility curves derived from different dogs during brief stimulations of the central vagus nerve with a strong current. They illustrate three types of curves which seem to characterize aortae with high, low, and intermediate degrees of elasticity.

In curve A the plots of systolic and diastolic $\frac{p}{d}$ relations exhibit almost a straight line function between pressures ranging from 80 to 172 mm. Hg. Such aortae, like those of all dead dogs studied, are highly elastic.

Curve B rises linearly between pressures of 60 to 110 mm. Hg and then bends toward the abscissae indicating reduction in distensibility above 110 or 115 mm. Hg. In curves of this type the reduction of distensibility at higher pressures is not sufficient however to cause a reversal of the diminishing effect that increased peripheral resistance has on pulse pressure.

Curve C likewise rises linearly up to a pressure of about 95 mm. Hg, but between 100–145 mm. Hg the distensibility reduces rapidly and above 150 mm. becomes practically a horizontal line. The lack of expansion at higher pressures was confirmed by observation and palpation; the aorta appeared like a rigid tube and no expansion could be felt by the finger. In such animals a reversal of a diminishing into an increasing pulse pressure may be anticipated.

On the basis of many such observations on 11 dogs we concluded that, during life, the distensibility of the aorta under high internal pressures

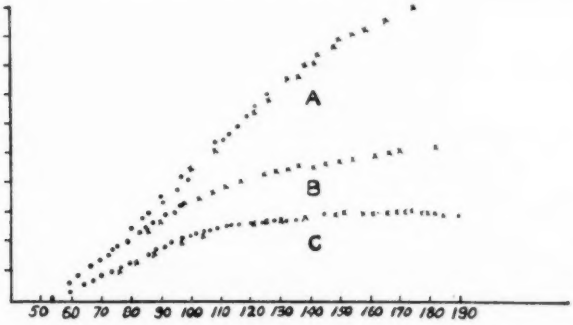

Fig. 1

varies in different animals. We hesitate to relate this solely to the ages of dogs which we hardly feel competent to estimate by criteria commonly employed to determine canine ages. Without denying such an age factor we are inclined to believe it is not the only one. For example, the linear distensibility curve published as part of figure 2 in a preceding article (7) was that of the aorta from a dog that yielded curve C of figure 1 during life. Furthermore, we discovered that while systolic and diastolic diameters determined during the rise of pressure fall on the same line, similar points determined during the subsequent decline of pressure often failed to do so. This in fact led to the study of changes in aortic diameters during somewhat more prolonged hypertension.

The effects of acute nervous hypertension. By this term we characterize hypertension induced by simultaneous stimulation of both vagi nerves with strong faradic currents. By this procedure pressures rise to higher levels and can be held there for at least 30 seconds. Thus in one experi-

ment the pressures rose within 15 seconds from 74/58 to 270/208 mm. Hg, the pulse pressure increasing from 16 to 62 mm. and, after remaining at this level for 12 seconds, gradually fell and ultimately returned to 72/62 mm. Hg. The data are plotted in the upper curve of figure 2 using ascending and descending pressure abscissae. The distensibility curve indicates an aorta with intermediate elasticity, diminution of expansion beginning at 100 mm. Hg. The important feature of this and many other similar experiments is the diminishing size of the aorta above pressures of 180 mm. Hg illustrated from C and D of figure 2.[3] With cessation of stimulation at D arterial pressures decline reaching a level of 109/85 in 3 minutes, during which time the aorta continues much smaller than at corresponding pressures during the rise. This is illustrated between D and E in figure 2. As the pressures gradually return to low initial levels, the size of the aorta with respect to the pressures also reverts to normal levels as shown between E and F in figure 2. Such reactions could be obtained repeatedly

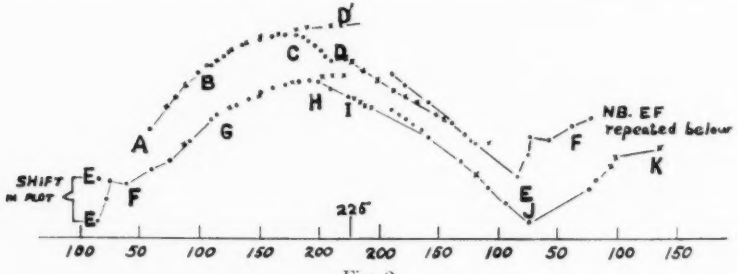

Fig. 2

in the same animals, once as many as seven times. The second curve in figure 2 extending from F to J represents the result following a second stimulation of both central vagus nerves. It is plotted at lower ordinate levels in order to separate the curves. The behavior of the artery was nearly identical with that following the first stimulation. Reactions such as these were obtained 22 times in 9 dogs; they failed to occur only 3 times in 2 different animals whose aortae appeared to belong to very elastic types.

Stimulation of the sinus nerves by clamping both common carotid arteries showed similar but smaller responses; in other animals in which vagus stimulation caused a decrease in size, carotid clamping was without effect. However in these experiments high pressures did not result.

Two possibilities of technical faults must be eliminated before it can be accepted that the aortic diameter actually decreased as seems to be

[3] The natural trend, plotted from earlier systolic $\frac{p}{d}$ relations is indicated by curves (X) from C to D'.

indicated by aortagrams. They are *a*, the possible shifting of the lever attachments so that they become located at points that do not remain in line with the axis as the artery expands, and *b*, the possible occurrence of small leaks in the recorder or aortagraph. The first error was excluded as far as possible by repeated observations during the tests and would not cause larger arterial excursions with identical pulse pressures as is illustrated by comparison of beats Y and Z of figure 4. The second possibility of error was rigorously checked in various ways. The arteriograph and segment capsule were covered by special rubber and the systems tested under positive and negative pressures before and after each experiment. The possibility that leaks occurred is excluded on other bases. It seems unlikely that different rubbers on different days could *all* develop leaks *after* tests had been made. On several occasions the same apparatus proved to be air-tight for a number of days. Finally, while a leak could explain the descent of the aortagrams and possibly the recovery, it could

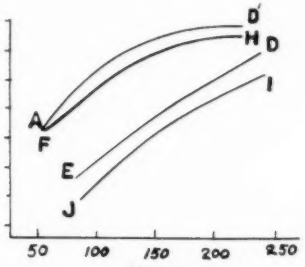

Fig. 3

not account for coincident changes in the distensibility now to be discussed.

Measurements of plots such as those of figure 2 show that the slopes of the distensibility curves during rising and receding pressures (cf. A,D' and D,E; or FH and IJ) are quite different. The character of the changes is seen at a glance when smoothed curves are replotted over the same abscissae as in figure 3. There is no question that with reduction in size the distensibility curves (E,D and J,I) become more nearly straight-line functions after a brief operation of reflex hypertension than during its development as indicated by curves A,D' and F,H. This enhancement of distensibility operates particularly above 100 mm. Hg and results in volume-elasticity relations that correspond essentially to those in dogs that have died recently (7). Such results obtained repeatedly in numerous experiments strongly suggest that both the size and distensibility of the aorta is capable of changing as a result of afferent nerve stimulation.

A number of tests were made to determine whether efferent nervous mechanisms were concerned. To this end, the aorta was painted either

with procaine or cocaine solution previous to the production of nervous hypertension. Satisfactory conclusions could not be formulated since all the tests were made toward the end of long experiments and often after use of several chemical agents.

The effects of acute hypertension due to humoral agents. Small doses of adrenalin (e.g., 1.5 cc., 1:50,000 sol.) given slowly by vein to vagotomized dogs generally cause an acute hypertension more rapid in development, similar in magnitude, but shorter in duration than that due to vagal reflexes. The effects on aortic size during the rise and recedence of pressures depicted in graphic records are similar to those experienced in nervous hypertension, except that the diminution in size develops more rapidly. In order to emphasize the effects of augmented distensibility produced by adrenalin the changes are illustrated in figure 4 by segments of original records, rather than by plots. As shown in segment A pres-

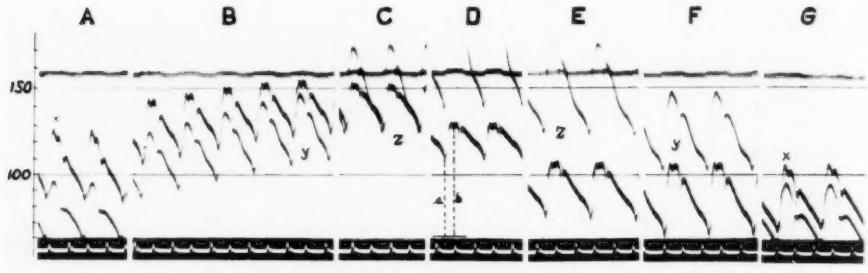

Fig. 4. Aortic pressure curves (lower curves in A, B, C, G; upper curves in D, E, F) and aortagrams of thoracic aorta (upper curves in A, B, C, G; lower curves in D, E, F). Time 1/5 sec.; ordinates apply to pressure curves.

sures start at low levels but rapidly mount after injection of adrenalin as shown in segments B and C. With this increase in pressures, the aortagrams also rise indicating that the aorta progressively expands. A plot of systolic and diastolic diameters in relation to internal pressure reveals an aorta of intermediate elasticity. At the summit of pressures, reached in segment D, the size of the aorta begins to decrease as evidenced by the drop of the aortagram. As the effects of adrenalin on the peripheral vessels and heart gradually wear off (E,F) the systolic and diastolic sizes become still smaller. That the size of the aorta decreased actively can easily be seen by comparing the pairs of beats marked *y* and *z* (in segments B,C, E,F) in which the diastolic pressures were nearly the same. Within a few minutes after pressures have practically returned to normal levels, the aorta gradually expands again until normal relations are reëstablished. Comparison of segments G with A shows an approximate recovery. Complete recovery was found in two other tests on this animal.

As in nervous hypertension, the aorta not only diminishes in size but its distensibility increases. The effects on the systolic excursions are well shown in these records. During the period of rising pressures (A,B) the systolic expansion decreases as the pulse pressure increases. In segments C and D an enormous increase in pulse pressure is not accompanied by a further increase in systolic expansion. In fact, as contraction of the artery begins to be indicated in segment D the expansion is a trifle less, despite the enormous pulse pressure. These effects illustrate the effects of diminishing distensibility at higher pressures. With the decline of pressures something happens to alter these relations. With gradually reducing pulse pressures manifested in segments E and F the expansions become much greater than at equivalent pressures and pulse pressures shown in segments B and C. This is specifically shown by comparing equivalent beats *y* (segments B and F) or similar beats *z* (segments C and E). In these, not only the diastolic pressure but the pulse pressures happen to be essentially the same. During the waning of adrenalin effects the aortagram excursions are much larger for corresponding pressure variations. It is difficult to explain such a greater expansion of a narrower artery without the assumption of an increased distensibility.

Indications of a similar decrease in caliber and change in extensibility of the aorta during acute hypertension were also obtained through action of pitressin and asphyxia. Pitressin acts to cause a more prolonged state of contraction while the effects were barely indicated during asphyxia.

Discussion. Graphic evidence indicates that during acute hypertension due to nervous and hormonal agents the aorta first expands and thereafter diminishes in size. This effect has to our knowledge not hitherto been observed. While the aorta decreases in diameter, its distensibility increases. This is in accord with previous observations on central vessels by Hürthle (3) and also by Wezler (5). Whether these changes are due to nervous, mechanical, or chemical stimulation was not proved.

Both processes presuppose some active change in the vessel wall, possibly physical alteration of the elastic elements, but more probably a muscular action. By the latter, either one of the phenomena could be explained; but it is hazardous to attribute both to the same process until we are better informed regarding the effects of muscular contraction. Two possibilities exist: on the basis of ring experiments, it seems that muscular contractions produce a decrease in diameter and in lumen. (For refs. see 2.) According to the histological studies of Benninghoff (2) and the circumstantial evidence of Wezler and Böger (5) contraction acts chiefly to release tension on elastic fibers, thereby increasing their distensibility. Our results might be interpreted as evidence that both processes are induced were it not for the observations that the increase in distensibility is usually not synchronous with the decrease in size but generally occurs a

little later. For the present we therefore prefer to record the facts and avoid further speculation regarding the mechanisms.

Dynamically, decrease in diameter and increase in distensibility act oppositely as far as modification of pulse pressure is concerned. Decrease in volume like augmented output of the heart tends to augment pulse pressure; increase in elasticity of a degree shown in figure 3 tends to reduce pulse pressure. It is difficult to evaluate the resultant of these forces on pulse pressure during our experiments. Even a guess is precluded because the importance of measuring the initial diameter of the aorta was not recognized at the time the experiments were performed. While the possibility cannot be excluded that changes in volume of the central vessels increase pulse pressure during nervous and hormonal hypertensions, the fact that such significant changes in volume and elasticity occur is a discovery worthy of being reported.

If similar changes occur in man, they can certainly not be disregarded in the evaluation of central pulse velocities. This may be one reason for the existence of such variable reports of central pulse velocities in human subjects with hypertension.

SUMMARY

1. The expansion of the thoracic aorta was studied by means of an optically recording aortagraph during the development and recedence of acute hypertension, produced by nervous and hormonal means.

2. Distensibility curves plotted from consecutive aortagrams and corresponding optical pressure curves (recorded by a calibrated manometer) indicate that dogs' aortae fall into three groups, viz., those with high, low and intermediate degrees of distensibility. In the first two groups the aorta was definitely less distensible during life than shortly after death. The reason for this is not clear.

3. Except in aortae classed as having low degrees of distensibility, a reversal of diminishing into increasing pulse pressures with rising pressures caused by increased peripheral resistance is not probable on the basis of physical studies recently reported (7).

4. During the rise and recession of pressures in acute hypertension produced by nervous and hormonal agents, the initial passive expansion is followed by an active diminution in size which outlasts the peripheral constriction, but from which recovery occurs in 3 to 5 minutes after pressures have returned to lower levels. The exciting agent and ultimate mechanisms remain to be determined but the natural conclusion would be that muscular contraction is involved.

5. The diminution in size of the thoracic aorta is followed after a definite latent interval by increased distensibility of the walls. Whether this is

an effect of the same muscular action that causes diminution in size or to some other obscure mechanism cannot be decided at present.

6. Dynamically, decrease in volume and increase in extensibility act as opposites in determining the pulse pressure amplitude and height of systolic pressures. Whether they neutralize each other or whether one dominates cannot be determined by data available.

REFERENCES

(1) Atzler, E. and G. Lehmann. Bethe Handb. der Physiol. VII/2, 964, 1927.
(2) Benninghoff, A. Handbuch der mikroscop. Anat. des Menschen VI, 1930.
(3) Hürthle, K. Pflüger's Arch. **200:** 49, 1923.
(4) Tennant, R. and C. J. Wiggers. This Journal **102:** 351, 1935.
(5) Wezler, K. and A. Böger. Arch. f. exper. Path. u. Pharmakol. **180:** 381, 401, 1935–36.
(6) Wiggers, C. J. This Journal **40:** 219, 1916.
(7) Wiggers, C. J. This Journal **123:** 644 1938

THE VITAL IMPORTANCE OF SALIVARY GLANDS TO NEWBORN RATS[1]

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In the course of experimental surgery in very young rats on another problem it became apparent that removal of certain parts of the salivary gland complex was invariably followed by death. During an attempt to gain an explanation for this unexpected result a large group of young rats has been subjected to removal of certain salivary glands, closure of salivary ducts and various combinations of these. Preliminary findings of this attempt were published briefly (Plagge, 1937). These results and further investigations are recorded in detail in this paper.[2]

MATERIALS AND METHODS. Two-hundred and seventy-seven albino rats, representing 45 litters ranging in age from birth to 20 days, have been operated in these experiments. Ether anesthesia was used in all, except for a few cases of newborn rats in which anesthesia by refrigeration was used (Taylor and Wiesner, 1932a).

The closely associated submaxillary and major sublingual glands, the only salivary glands involved in the present experiment, were approached by a mid-ventral incision of the skin in the neck region. Small operating instruments, a binocular dissecting microscope and strong lights made it possible to locate not only the ducts of the glands in newborn rats but also principal blood vessels.

A gland was removed only after its duct had been either ligated or traumatized. In instances where the glands were left intact but their ducts were ligated, a small segment of the duct was removed to eliminate the possibility that its ends might later unite.

In all operated controls the glands were lifted completely from the neck and left hanging freely by their ducts and blood vessels outside the body for a period of time equaling that required to perform an operation.

[1] This investigation has been aided by a grant from the Rockefeller Foundation to The University of Chicago.

[2] The author wishes to take this opportunity to express his sincere appreciation to Prof. Carl R. Moore for his generous advice during the course of this work and particularly in reference to the preparation of this paper for publication; to Prof. Robert R. Bensley for his friendly interest and criticisms; and to Dr. Jule K. Lamar for suggestions and assistance during the course of the investigation.

Young operated rats were observed daily, or in some instances, at half day intervals. Body weights were recorded to the nearest one-hundredth of a gram, and the rats were inspected for milk in their stomachs which is easily seen through the abdominal wall of young rats. Finally, operated animals were autopsied.

TABLE 1

Effects of salivary gland removal on young albino rats

EXPERIMENTALS						CONTROLS			
Total number of rats	Age at operation	Living		Dying		Not operated		Operated	
		Number	Body weight (% of normal)	Number	Time of death (days post-op.)	Number	Number dying	Number	Number dying
1. Bilateral removal of both pairs of glands									
	days								
67	0–8	0		67	2–8	24	0	30	7
4	10	1	40	3	5–7	0		3	0
7	13–20	6	Retarded	1	5	4	0	4	0
2. Bilateral ligation or resection of ducts									
21	2–8	3	100	18	2–10	9	0	13	0
2	10	2	40	0		0		2	0
3	13–17	3	Retarded	0		1	0	2	0
3. Unilateral removal or duct ligation of both salivary glands									
9	2–16	9	100	0		7	0	3	0
4. Removal of both submaxillary glands									
8	3–19	8	100	0		6	0	3	0
5. Removal of both major sublingual glands									
5	3–5	5	100	0		5	0	1	0
6. Removal of all but one submaxillary or one major sublingual gland									
23	3–13	19	100	4	3–5	4	0	10	0

EXPERIMENTAL PROCEDURE. *Bilateral removal of submaxillary and major sublingual glands.* In order to determine the outcome of removal of two submaxillary and two major sublingual glands, operations were done on 78 rats with ages ranging from birth to twenty days. These were controlled by 28 other untreated members of the same litters and 37 which were subjected to control operations but not salivary gland removal. Data are presented in section 1 of table 1. All animals having both pairs of

glands removed prior to day 10 died within 8 days; 3 of 4 operated on day 10, and one out of 7 operated from days 13 to 20 died between 5 and 7 days post operation. The living animals were retarded in growth. Death of 7 operated controls was presumably due to over chilling in anesthesia.

It was observed through the ventral body wall that the stomachs of those experimental animals which died were very soon devoid of milk, and the animals gradually lost weight before death which was apparently due to inanition.

Bilateral ligation or resection of ducts of submaxillary and major sublingual glands. Since it became apparent from the above results that the submaxillary or major sublingual glands, or both were essential to life in young rats under 10 days of age, attention was directed to determining whether this involved an endocrine or an exocrine function.

To test this question, 21 rats, ranging in age from 2 to 8 days, were operated and the 4 ducts of the 4 salivary glands ligated (see 2, table 1). A normal circulation in duct-ligated glands has been confirmed not only by gross appearance at autopsy but also by histological examination. Eighteen of the 21 experimental rats died 2 to 10 days after the operation; their stomachs had been empty during the postoperative period. Autopsy of 2 of the remaining 3 rats that lived revealed normal intact salivary gland ducts on one side. This observation may indicate: 1, neglect in operating one side; 2, imperfect ligation of the ducts; or 3, occurrence of accessory ducts. The third rat that lived represents the only exception to the usual result in these experiments. It was one of 5 rats whose salivary gland ducts had been ligated at 3 days of age. Autopsy at 50 days showed degenerated salivary glands whose appearance was that of glands whose ducts have been successfully ligated for as long a period. The 21 rats in the last category had 22 controls, 13 of which were operated and all of which developed normally.

Five rats were operated at 10 to 17 days of age and the ducts bilaterally ligated. None of them died although their body weights were retarded, in some cases to 40 per cent normal. Five controls, 4 operated and one normal, grew normally.

It would seem from these disastrous results of bilateral ligation of all ducts leading from submaxillary and major sublingual glands in rats 2 to 8 days old, that the disturbance was of the external secretion rather than internal secretion.

Unilateral removal or duct ligation of submaxillary and major sublingual glands. Although normal growth of operated control animals suggests that the disturbing factor in rats from which the salivary glands have been removed is in the glands themselves, rather than in associated structures, further control was employed. Nine animals from 2 to 16 days of

age were subjected to unilateral removal or duct ligation of both glands (see 3, table 1). All operated animals lived and gained in body weight at the same rate as 10 controls, 3 of which were operated controls. Milk was observed in their stomachs at all times after operation.

There is no indication from these results that normal growth is disturbed by unilateral removal of submaxillary and major sublingual glands. In other words, the two remaining glands are sufficient to maintain the normal growth rate.

Removal of two submaxillary glands or two major sublingual glands. Having thus given evidence to indicate that removal of 2 submaxillary and of 2 major sublingual glands causes both loss in body weight and death when the operation is done before the rat is 10 days old, another consideration follows. Is the deficiency, whatever its nature may be, brought about by: 1, removal of submaxillary glands; 2, removal of major sublingual glands; or 3, removal of both submaxillary and major sublingual glands?

Submaxillary glands were removed from 8 rats, ranging in age from 3 to 19 days, leaving the two major sublingual glands (see 4, table 1). Although the gross weight of the major sublinguals is only about 15 to 20 per cent as great as that of the submaxillary glands, all animals lived and gained weight at a normal rate. Milk was seen in their stomachs at all times after operation. Nine controls, 3 of which were operated, developed normally.

The two major sublingual glands were removed from 5 animals, ranging in age from 3 to 5 days (see 5, table 1). The growth curve of each of these animals was normal as compared with one operated and 5 unoperated controls.

It would appear, therefore, that the observed interference with normal development resulting from salivary gland removal is not specifically related to either the major sublingual or the submaxillary glands. Young rats develop normally when either pair of glands is left intact but die when all four glands are removed.

Removal of all but one submaxillary or one major sublingual gland. Confirmation of the results obtained by removal of two submaxillary or two major sublingual glands was attempted by two types of operations: 1, all glands were removed except one submaxillary, and 2, all glands were removed except one major sublingual.

Twenty-three rats, ranging in age from 3 to 13 days, had only one submaxillary (10 rats) or one major sublingual gland (13 rats) left functionally intact after operation (see 6, table 1). The 10 rats with one submaxillary gland lived and developed normally.

The results for the remaining 13 rats which had only one major sub-

lingual gland were somewhat variable. Five of 9 animals so operated between 3 and 5 days developed normally. Other rats similarly operated at 8, 10, and 13 days, also lived.

The remaining 4 of the 9 animals operated between 3 and 5 days died in 3 to 5 days after the operation. In these cases the possibility of damage to the one remaining major sublingual gland during the operation when it was being separated from its firm adhesion to the submaxillary gland can not be overlooked. Furthermore, the ducts of these 2 glands run closely together in a common sheath. In the first attempts to distinguish and separate the ducts by dissection under a binocular microscope, a certain amount of uncertainty was felt in regard to the technique, and the data recorded here include even those preliminary operations which were not considered entirely satisfactory.

The observed inconsistency in results in a few cases, whether due to gland injury at operation or to actual inability of the remaining gland to produce enough substance to maintain life, is probably insignificant since the majority of animals with but one submaxillary or one major sublingual gland were not affected by removal of the other three glands.

There were 14 controls in this last category, 10 of which were operated and all of which developed normally.

A few experiments involving removal by a transverse cut of the half of these salivary glands distal to the duct did not influence the growth of young rats.

In summarizing these results one may conclude that rats less than 10 days old lived, had milk in their stomachs and gained in body weight at a rate comparable to that of normal or operated controls, providing any one of these 4 glands was left functionally intact. In contrast to these results, complete removal of all 4 salivary glands or ligation of their ducts resulted in empty stomachs, loss in body weight and death.

The investigation was continued to determine the nature of the function of these salivary glands. The problem has been approached by several types of experiments in an effort to determine whether death is caused *a*, by inanition; *b*, by a disturbance in the physiology of digestion; or *c*, by lack of effort on the part of the young to feed.

a. To compare results obtained through salivary gland removal with effects from total inanition, mammary occlusion was performed on the mother of a litter of 6 rats on the 4th day after delivery. This was accomplished by making a U-shaped incision around the base of all teats. Each U-shaped flap of skin with its teat was turned under and fastened securely to the under surface of the skin, and the sides of the incision were brought together by suture. The young, though unable to suckle, were provided with other normal nest conditions such as warmth, moisture, and general care from the mother. Two of these young were subjected to

complete removal of submaxillary and major sublingual glands; 2 served as operated controls without removal of tissue; and the remaining 2 served as unoperated controls. This group of 6 young died within 5 days after mammary occlusion. A second group of young subjected to salivary gland removal but whose mother was normal died in the same time. All young of the 2 groups failed to reveal milk in their stomachs. It is clear, therefore, that total inanition of normal young rats gives entirely similar results to those in which salivary glands have been removed.

b. To determine whether the results are due to disturbances in the physiology of digestion or to some mechanical interference with feeding, four-day-old operated rats were force-fed. A fine rubber catheter fastened to a hypodermic needle was forced down the esophagus and warmed cow's milk injected into the stomachs at 4-hour intervals day and night until the rats reached an age of 17 days. In a litter of 5 young, 4 were subjected to total salivary gland removal 4 days after birth and the 5th member served as the unoperated control. Beginning on the day of operation 2 of the 4 operated animals were force-fed. Five days after operation the 2 unfed operated animals died, weighing $5\frac{1}{2}$ grams each; the two force-fed animals weighed 11 grams each whereas the normal control weighed 17 grams. At 17 days the force-fed animals obtained food for themselves and continued to develop normally. It is evident, therefore, that the disturbance caused by salivary gland removal centers on the ability to obtain milk (suckling) rather than on the ability to carry on digestion alone.

c. To observe directly the feeding behavior of operated rats, 3 litters were employed in each of which one-half the animals suffered total salivary gland removal, while the other half were operated controls without removal. Each litter was confined in a wire mesh bottomed cage supported above a mirror; methylene blue served to mark the animals individually. The feeding activities of each rat were easily followed through reflections in the mirror. Frequent observations of approximately one-hour's duration revealed that, while experimental rats were usually actively engaged in an effort to suckle and frequently located teats, they were unable to attach themselves firmly. Their nervous feeding behavior was in marked contrast to that of the operated controls which usually suckled quietly at one teat for 5 to 10 minutes. The test animals dropped from the teats when the mother moved, whereas the operated controls held on so as to be dragged along. The contention, therefore that the mechanics of the ingestion of milk is disturbed in young rats after salivary gland removal is strengthened by direct feeding observations. Insufficient moisture in the oral cavity to enable the rat to attach its mouth firmly enough to its mother's teats to obtain milk is a possible explanation of death rather than one involving the physiology of digestion or an endocrine imbalance.

Histology. Ligation of the submaxillary and major sublingual gland

ducts of newborn or young rats results in rapid pressure degeneration of of the glands as revealed by histological study. Progressive histological changes have been studied beyond the fatal period in a series of animals operated unilaterally at the age of 4 days. Three or four days after duct ligation signs of degeneration can be detected by comparison with the glands on the unoperated side of the animal, while 10 days post-operation, by which time all bilaterally operated animals have died, degeneration has proceeded to the extent that the entire gland is much reduced in size, both large and small ducts are much distended with their epithelium reduced to a thin solid line, and large areas of parenchyma are replaced by connective tissue. At 26 days after duct ligation at 4 days (figs. 1 and 2) the reduced and degenerated organ is hardly recognizable as a salivary gland.

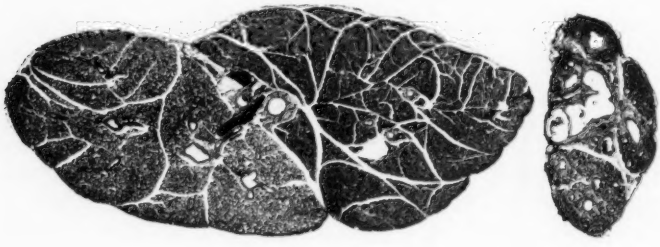

Fig. 1 Fig. 2

Effects of submaxillary and major sublingual duct ligation in a 4-day-old rat.
Fig. 1. Cross-section of normal glands at 30 days. (35 ×.)
Fig. 2. Salivary glands from opposite side of same animal as figure 1, showing effect of 26 days of duct ligation. (35 ×.)

Discussion. The facts established by this investigation, that total removal of the submaxillary and major sublingual glands from rats one to eight days after birth invariably results in death of the young, suggested at first an endocrine disturbance. To the writer's knowledge no data up to the present time have indicated such vital importance of this salivary complex. It is generally assumed that the principal function of the salivary glands is to moisten the food and so aid mastication and swallowing, but it is also known that a digestive function is exercised by the saliva at least in some animals. One can scarcely believe that in the young rat the mother's milk requires moistening or that added lubrication would be required for the mechanism of swallowing.

The fact that forced feeding of cow's milk after such salivary gland removal suffices to maintain life and permit growth but slightly below normal argues against the loss of an essential digestive substance secreted by the glands. The further fact that such operated animals fail to obtain

milk through their efforts to suckle either when all members of the litter are operated or when normal littermates are present points to an interference of some nature either with nipple attachment or the act of suckling or swallowing.

The mirror reflections clearly revealed attempts at suckling, and the fact that operated young became attached to the nipple though insecurely suggests some interference with muscular function in the act of suckling or swallowing. It is of interest to recall that any one gland of the complex of four remaining intact permits normal growth. Furthermore since operations involving complete dissection of all glands to the extent that they hang over the sides of the incision by their ducts alone are harmless, and since less vigorous dissection accompanied by ligation of the ducts is fatal, it would seem to be established that it is actually the loss of secretion from these glands and not mere surgical trauma that is the cause of the disturbance.

It is of further interest to realize that salivary gland removal subsequent to the 10th day of life exerts much less influence since such animals can live and gain. Body growth, however, is retarded by comparison with controls, the effect being most pronounced the younger the animal at the time of operation. Finally, if the salivary glands are not removed until the rat is 20 days old or older, development is identical with that of operated littermate controls.

CONCLUSIONS

1. Newborn rats deprived of the two submaxillary and two major sublingual glands invariably die within 5 days.
2. Any one member of the pair of 4 glands suffices for continued life and growth.
3. Ligation of the salivary ducts likewise results in death of the young, and histological study reveals rapid pressure degeneration of the glands.
4. Death from salivary gland removal is due to inanition since the young, though making attempts to suckle, fail to swallow milk.
5. Operated young force-fed with cow's milk survive and grow.
6. It appears that the disturbance is associated with some mechanical factor in the feeding process rather than with some chemical phase of the physiology of digestion.

REFERENCES

PLAGGE, J. C. Anat. Rec. **70** (suppl.): 60, 1937.
TAYLOR, H. AND B. P. WIESNER. J. Physiol. **75**: 39 P, 1932a.

THE BLOOD PICTURE OF RATS FROM BIRTH TO TWENTY-FOUR DAYS OF AGE

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A survey of the literature (Addison and Richter, 1923; Briese, 1936; Chisolm, 1911; Drabkin and Fitz-Hugh, 1934; Enzmann, 1934; Fitz-Hugh, Robson and Drabkin, 1933; Jolly, 1906, 1909; Kindred and Corey, 1930; Orten and Smith, 1934; Smith, 1932; Sure, Kik and Walker, 1929; Wigodsky and Ivy, 1938; Wills and Mehta, 1930; Wintrobe and Shumacker, 1935, 1936) describing one or more of the components of the blood picture of rats from birth to twenty-four days of age indicated the inadequacy of data for the purpose of evaluating statistically the effects of experimental procedures on such animals. Moreover, the not inconsiderable differences between the reported values suggest the possibility of technical and colony differences. It is the purpose of this report to present a controlled set of daily observations on the blood picture of rats of the above age group, with emphasis on the determination of the degree of variability, thereby delineating statistically significant values.

MATERIALS AND PROCEDURE. The parents of the young rats were of the University of Chicago pied-albino stock, and weighed 250 or more grams. They were kept in breeding cages until near parturition and then isolated. The young remained in litter groups until used. One-half of the mothers received, *ad libitum*, a diet of boiled beef lung-bread-milk and a mixture of Klim and cornmeal. The other half received only Purina checkers.

The observations were made on 288 young rats, twelve for each day, from birth to twenty-four days of age. Rats 0 to 24 hours old were classed as one day old; those 24 to 48 hours old, two days old, etc. At least eight of the twelve rats for each day were from different litters. Seventy-six litters were sampled. As far as possible it was attempted to space the young of a litter over the entire twenty-four days. The young were not grouped according to sex. The data were collected over a period of eleven months. The weight and length of the young agreed with data collected by Donaldson (1924).

Blood was collected by clipping off the head and allowing the body to bleed into a dry, heparin-prepared vessel. The blood values determined

were erythrocyte count, hemoglobin estimation, volume of packed erythrocytes, reticulocyte percentage, and leucocyte count. Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (Wintrobe, 1932) and absolute number of reticulocytes were calculated. The erythrocyte count was the average count of 1/25 sq. mm. in each of two separately filled chambers.[1] Hemoglobin was estimated by the Newcomer method. The volume of packed erythrocytes was determined by use of Van Allen hematocrit tubes. The reticulocyte percentage was derived from a count of 1000 cells of the red cell series by the wet mount technique (Wakerlin, Bruner and Kinsman, 1936); all cells showing formed, vitally stained bodies were considered reticulocytes. The leucocyte count was the average count of 8 sq. mm. Smears were stained with Wright's stain and examined for the presence of immature cell forms.

The error of observation derived from a statistical analysis of ten determinations on a single blood sample is presented in table 1. Data derived from fifty-two counts on twenty-one normal, adult, female rats of the same strain and by the same technique are presented in table 2 for purposes of comparison.

Results. The observations on the 288 young rats enumerated above are condensed and summarized in table 3. From the work sheet only the means ($\overline{X}$) and the standard deviations (σ) are presented; from these values, however, most of the usual statistical values may be derived. Of the 2,592 separate determinations comprising table 3, only two (0.08 per cent) are beyond the limits of $\overline{X} \pm 3\sigma$. The coefficients of variation of the values in table 3 are larger in 99.5 per cent of instances than the corresponding coefficients of variation in table 1.

As seen in both the wet mounts and the dry smears, the erythrocytes, particularly of the younger rats, exhibited wide variations in size. Poikilocytosis and polychromatophilia were apparent only in preparations from rats less than six days old. The high reticulocyte counts of the first four days were due to the large proportion of cells containing small, discrete, vitally staining particles. At the lower level of 20 per cent to 30 per cent the reticulocytes were the usual rod, reticulum, or flake type. Frequently the reticulum, as seen in wet mounts, was so dense as to suggest the condensed nucleus of the normoblast but immature forms were seen very infrequently in the dry smears; basophilic stippling was never observed in the dry smears.

Litter mates exhibited variations in blood picture as large as those among unrelated animals of the same age.

The possible correlations of the means in table 3, except those which

[1] Red and white cell pipettes and brightline hemacytometer were certified by the United States Bureau of Standards.

reduce to a primary set of observations or reciprocals, were tested, but were not judged significant.

By use of the method of running averages, it is found that the erythrocytes steadily increase at the rate of approximately 100,000 per c.mm. per day. The mean corpuscular volume and the mean corpuscular hemoglobin decrease at a fairly constant rate up to the eighteenth day. From the

TABLE 1

Statistical analysis of 10 determinations on one blood sample indicating error of observation

	R.B.C.*	Hb.*	Ht.*	M.C.V.*	M.C.Hb.*	M.C.Hb.C.*	RETICULOCYTES*		W.B.C.*
							Per cent	Absolute	
Mean	2.720	7.18	25.25	92.90	26.44	28.46	16.6	0.451	3.395
Standard deviation	0.106	0.174	00.50	4.70	1.20	0.27	1.34	0.0012	0.449
Coefficient of variation (per cent)	3.89	2.40	1.98	5.06	4.50	0.95	8.07	0.27	3.20

* R.B.C. = Erythrocytes in millions per c.mm.; Hb. = Hemoglobin in grams per 100 cc. blood; Ht. = Per cent packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in per cent; Reticulocytes expressed in per cent erythrocytes and absolute in millions per c.mm.; W.B.C. = Leucocytes in thousands per c.mm.

TABLE 2

Statistical analysis of 52 counts on 21 normal adult female rats

	R.B.C.*	Hb.*	Ht.*	M.C.V.*	M.C.Hb.*	M.C.Hb.C.*	RETICULOCYTES*		W.B.C.*
							Per cent	Absolute	
Mean	9.422	14.90	46.44	49.7	16.3	32.7	2.6	0.226	13.476
Standard deviation	0.508	2.54	7.72	3.90	1.31	1.91	2.00	0.156	7.280
Coefficient of variation (per cent)	5.39	17.04	16.62	7.8	8.1	5.2	76.9	69.5	54.0

* See footnote, table 1.

eighteenth to the twenty-fourth day the means of these two values (table 3) hold levels higher than the means of the corresponding adult values (table 2). The running averages of the hemoglobin and hematocrit show a decrease for the first eight days; a plateau for six days is followed by another drop; then a definite rise begins at the eighteenth day. It is noteworthy that about the eighteenth day the mothers showed a disinclination to nurse and the young became more active and began to forage. The reticulocytes

drop from a high of 88.8 per cent at birth to 30.1 per cent on the fourth day and vary between 19 per cent and 32 per cent during the remaining twenty days. Individual variations of this determination are large, as indicated by the mean coefficient of variation of 25.4 per cent. The mean absolute number of reticulocytes decreases from 2,000,000 per c.mm. to about 1,000,000 per c.mm. on the fourth day, a level which is then held with small variations. The mean corpuscular hemoglobin concentration gradually decreases from 31.5 per cent to 28 per cent during the twenty-

TABLE 3

Means ($\bar{X}$) *and standard deviations* (σ) *of blood values of 288 young rats*

(12 rats each day, from birth to 24 days of age)

AGE	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C. Hb.C.*		RETICULOCYTES*				W.B.C.*	
													Per cent		Absolute			
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
days																		
1	2.400	0.401	10.31	1.58	32.89	5.60	134.6	10.9	43.9	3.6	31.7	2.9	88.8	3.5	2.041	0.354	4.65	2.28
2	2.420	0.296	10.06	1.33	32.13	3.48	132.9	10.8	41.8	5.1	31.5	2.2	79.4	9.6	1.934	0.436	3.95	1.44
3	2.560	0.349	10.39	1.43	33.12	3.43	130.5	9.8	39.9	3.7	31.3	1.8	49.3	22.6	1.264	0.631	4.10	2.46
4	2.720	0.477	10.72	2.06	32.56	4.45	120.7	9.8	38.4	4.2	31.8	3.0	33.2	17.2	0.915	0.551	4.00	1.51
5	2.640	0.325	9.92	1.41	32.00	3.97	121.2	12.5	37.7	4.4	31.5	1.3	30.1	7.6	0.804	0.179	3.65	1.09
6	2.710	0.525	9.21	1.74	30.31	4.15	113.8	15.6	34.6	4.8	30.4	1.9	23.4	5.3	0.639	0.202	4.50	2.92
7	2.685	0.351	8.74	0.75	29.21	1.70	111.0	12.3	33.3	5.4	29.9	2.0	29.0	6.8	0.785	0.241	3.75	1.40
8	2.890	0.551	8.39	0.73	28.77	2.48	101.4	11.5	29.5	3.8	29.2	1.8	33.2	10.8	0.943	0.291	3.80	1.17
9	3.190	0.541	8.25	0.99	27.60	2.68	88.6	13.9	26.2	3.8	29.8	2.0	28.8	12.0	0.909	0.275	3.70	0.59
10	3.070	0.627	8.01	0.91	26.92	3.34	89.2	9.4	26.6	3.0	29.5	1.8	29.4	7.5	0.869	0.180	3.90	1.06
11	3.385	0.548	8.31	1.07	28.90	4.69	85.2	7.2	24.9	1.6	29.0	1.4	31.6	7.6	1.095	0.357	3.30	1.13
12	3.125	0.393	8.01	0.99	26.60	3.79	85.7	8.2	25.7	2.5	30.1	2.0	33.2	10.1	1.047	0.365	4.00	1.35
13	3.410	0.378	7.71	0.83	27.06	3.12	79.5	6.0	22.7	1.6	28.6	2.0	31.0	5.5	1.053	0.198	3.75	1.11
14	3.840	0.597	8.47	0.81	28.04	2.49	73.4	6.0	22.2	1.7	30.2	1.2	23.5	6.3	0.900	0.281	4.85	0.60
15	3.800	0.517	8.20	1.09	26.79	3.40	70.9	6.5	22.5	1.7	30.7	1.8	24.2	5.4	0.897	0.185	4.55	1.13
16	3.565	0.588	7.43	0.86	24.79	4.07	68.8	3.7	21.0	1.6	30.3	2.3	25.1	8.6	0.844	0.200	4.35	0.78
17	3.980	0.527	7.14	0.68	24.54	2.91	62.0	5.3	18.1	1.7	30.1	2.1	21.7	4.9	0.827	0.220	4.65	1.31
18	3.845	0.595	7.23	1.08	23.90	3.55	62.5	5.9	18.9	2.2	30.3	1.8	21.1	5.6	0.799	0.209	3.95	0.99
19	4.470	0.377	8.04	1.37	23.42	5.25	59.7	5.4	17.9	2.1	29.9	1.5	21.5	5.2	0.909	0.197	4.85	1.05
20	4.245	0.459	7.54	0.62	25.71	1.97	60.8	5.1	17.9	2.1	29.3	1.9	18.8	3.7	0.794	0.154	4.35	1.26
21	4.580	0.564	8.03	1.02	27.69	3.70	60.6	5.2	18.0	2.2	29.1	1.7	22.7	4.7	1.034	0.273	5.15	1.52
22	4.390	0.603	7.45	1.19	26.44	3.56	60.6	6.1	17.0	1.9	28.0	1.3	23.6	5.9	1.057	0.289	4.85	1.38
23	4.675	0.417	8.17	0.95	28.88	3.89	62.1	7.3	17.6	2.4	28.3	1.3	26.8	4.7	1.253	0.257	3.85	0.82
24	5.040	0.727	8.52	1.25	30.13	5.05	59.8	4.8	17.0	1.4	28.4	1.1	23.2	5.6	1.166	0.351	5.45	1.69

* See footnote, table 1.

four day period. The leucocytes slowly increase by approximately 1,000 per c.mm. during the period of observation.

By use of the standard error of two means method, it was found that the differences between the corresponding mean values of adults (table 2) and young at 24 days (table 3) were not attributable to chance, except in the instance of the mean corpuscular hemoglobin. Although the ranges of the mean corpuscular volumes of adult and young overlap, the difference between the means is real.

No significant differences were observed in the blood pictures of the young of mothers fed the two different diets.

Discussion. The degree of variability or scatter, as expressed by the standard deviation from the mean, is proportionally large and markedly broadens the limit of non-significance ($\overline{X} \pm 3\ \sigma$). In most instances the wide boundaries of non-significance on one day include the means of ten days before or after. It will be observed that only a small part of the variability may be ascribed to technique. Inasmuch as the young do not grow at the same rate, it is to be expected that blood formation in the different individuals will proceed at different rates. Chisolm (1911) has published data showing that in growing rats, from eight to twenty-one days of age, the blood volume increase lags behind the body weight increase. The data presented here show that the per cent of erythrocytes in the blood during this period is decreasing while the number of erythrocytes per cubic millimeter is steadily increasing. Thus, it is not difficult to understand how slight variations of erythropoietic rate in individual animals may result in marked variations from the calculated mean. A larger daily population would have minimized the calculated degree of variation.

Because of these considerations, only means, which are not too sharply delineated, and trends may safely be recognized. The trends of the data presented agree with those of the previously cited reports. A few of the published means for a given age agree with the means of table 3; the remainder, however, usually fall within the calculated non-significant zone. Probable reasons for these differences are not apparent, except in the instance of hemoglobin. Mitchell (1932) has concluded that the diet and parity of the pregnant mother rat may affect the hemoglobin per 100 cc. of blood of the young. Sure and Kik (1929) have shown that the diet of the lactating mother may affect the rate of hemoglobin production in the young. Drabkin and Fitz-Hugh (1934) suggest that the changes in the blood picture from birth to maturity may be related to the nutritional background and they call attention to "the existence of physiological differences" in the rat. As was earlier stated, no differences were observed in the blood pictures of the young of mothers on two different diets. While the diet may account for some of the discrepancies between the means in the literature and those presented here, the possibility of peculiar colony differences should not be discarded.

Wintrobe and Shumacker (1935, 1936) and Smith (1932) have pointed out the essential similarity between the blood picture of fetal and infant rats and the blood picture of pernicious anemia in man. Wintrobe and Shumacker (1935) further have shown the resemblances in the blood picture changes toward adult normal levels as observed in rat fetuses and newborn, and in man with macrocytic hyperchromic anemia after adequate liver therapy. The data presented here confirm these comparisons and,

in addition, point out that the gradually decreasing mean corpuscular hemoglobin concentration is like that observed during induced remission of pernicious anemia (Wintrobe, 1931). A dissimilarity, however, is observed in the behavior of the reticulocytes which remain at a high level despite the fact that the erythrocytes per cubic millimeter are constantly increasing.

According to one cytologic view, as concisely expressed by Jones (1938) and by Kato (1935), megaloblastic erythropoiesis is the normal process in the fetus and in man with pernicious anemia, whereas normoblastic erythropoiesis is the normal process in the normal adult. The transition from megaloblastic erythropoiesis of the newborn rat to normoblastic erythropoiesis of the adult rat is reflected in the data presented, particularly in the mean corpuscular volume and the mean corpuscular hemoglobin. Analysis of the curves of these two corpuscular values and of the reticulocytes strongly suggests the presence of a "degeneration" process. The data of table 3 are interpreted as representing the composite of the decline of one type of erythropoiesis and the substitution of another. This interpretation is wholly consistent with the high degree of variation previously noted. Studies of the blood of fetal rats (Kindred and Corey, 1930, 1931; Wintrobe and Shumacker, 1935, 1936) indicate that the substitution probably begins before birth. This study indicates that the substitution is cytologically complete or nearly complete by the eighteenth day after birth, although the mean corpuscular volume after that time remains at a mean level (for six days at least) significantly above the adult normal. This inconsistency may be explained by the report of Wintrobe (1934) that the presence of a reticulocytosis raises the mean corpuscular volume. Further support for this view lies in the observation (Smith, 1932) that the reticulocytes and the corpuscular size decline simultaneously to adult levels. An alternate explanation is that sufficient megalocytes survive for a time in the circulating blood to raise the mean volume of the red cell (Jones, 1938). Numerically, the adult blood values are not attained until after the fiftieth or sixtieth day of age, as is shown by other studies of some of these values (Drabkin and Fitz-Hugh, 1934; Fitz-Hugh, Robson and Drabkin, 1933; Orten and Smith, 1934; Sure, Kik and Walker, 1929; Williamson and Ets, 1926; Wintrobe and Shumacker, 1935, 1936).

SUMMARY

1. The blood values of 288 young rats, observed in groups of twelve for each day from birth to twenty-four days of age, are recorded.

2. Observations carried out on the bloods were erythrocyte count, hemoglobin, per cent packed erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes (per cent and absolute), and leucocyte count.

3. The marked variability from the mean of these blood values is noted.

4. The essential similarity in the blood pictures of the young rat and of pernicious anemia is emphasized.

5. The changes occurring in rats' blood during the period of observation, are interpreted to be the result of transition from megaloblastic to normoblastic erythropoiesis.

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REFERENCES

ADDISON, W. H. F. AND M. N. RICHTER. Quart. J. Exper. Physiol. (International Physiol. Congress XI) Suppl. p. 46, 1923.
BRIESE, E. Proc. Staff. Mayo Clin. **11:** 233, 1936.
CHISOLM, R. A. Quart. J. Exper. Physiol. **4:** 207, 1911.
DONALDSON, H. H. The Rat. 2nd ed. Philadelphia, Wistar Institute, 1924.
DRABKIN, D. L. AND T. FITZ-HUGH, JR. This Journal **108:** 61, 1934.
ENZMANN, E. V. This Journal **108:** 373, 1934.
FITZ-HUGH, T., JR., G. M. ROBSON AND D. L. DRABKIN. J. Biol. Chem. **103:** 617, 1933.
JOLLY, J. Compt. rend. Soc. de Biol. **60:** 564, 1906.
Compt. rend. Soc. de Biol. **66:** 136, 1909.
JONES, O. P. Proc. Soc. Exper. Biol. and Med. **38:** 222, 1938.
KATO, K. J. Lab. and Clin. Med. **20:** 1243, 1935.
KINDRED, J. E. AND E. L. COREY. Anat. Rec. **47:** 213, 1930.
Physiol. Zool. **4:** 294, 1931.
MITCHELL, H. S. This Journal **101:** 503, 1932.
ORTEN, J. M. AND A. H. SMITH. This Journal **108:** 66, 1934.
SMITH, C. J. Path. and Bact. **35:** 717, 1932.
SURE, B. AND M. C. KIK. Proc. Soc. Exper. Biol. and Med. **26:** 603, 1929.
SURE, B., M. C. KIK AND D. J. WALKER. J. Nutrition **1:** 299, 1929.
WAKERLIN, G. E., H. D. BRUNER AND J. M. KINSMAN. J. Pharmacol. and Exper. Therap. **58:** 1, 1936.
WIGODSKY, H. S. AND A. C. IVY. Proc. Soc. Exper. Biol. and Med. **38:** 787, 1938.
WILLIAMSON, C. S. AND H. N. ETS. This Journal **77:** 480, 1926.
WILLS, L. AND M. M. MEHTA. Indian J. Med. Research **18:** 307, 1930.
WINTROBE, M. M. Am. J. Med. Sc. **181:** 217, 1931.
J. Lab. and Clin. Med. **17:** 899, 1932.
J. Clin. Investigation **13:** 699, 1934.
WINTROBE, M. M. AND H. B. SHUMACKER, JR. J. Clin. Investigation **14:** 837, 1935.
Am. J. Anat. **58:** 313, 1936.

THE TIME FOR THE OCCURRENCE OF CORTICO-ADRENAL HYPERTROPHY IN RATS DURING CONTINUED WORK

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It is well known that the adrenal glands gain in weight when an animal is maintained under any type of stress. The object of this experiment was to determine how soon after the beginning of a stress a response in weight of the adrenal glands of rats could be detected and to determine whether the extent of the response in weight could be modified by 1, administration of cortin; 2, hypophysectomy, or 3, administration of the adrenotropic hormone. For the production of a constant state of stress the animals were anesthetized and the gastrocnemius muscle was stimulated to work. The effects of anesthesia, muscular work and inanition all contributed to the general state of stress.

METHODS. The animals were male rats of the Wistar strain having a range in body weight of 180 to 185 grams. Hypophysectomy was carried out under ether anesthesia by the usual ventral parapharyngeal approach. The cortin used was a standardized preparation of which each cubic centimeter represented 75 grams of whole adrenal gland. A commercial preparation of the adrenotropic hormone was used. On the basis of assay tests conducted by me it was shown to have the property of preventing atrophy of the adrenal cortices of hypophysectomized male rats (180 to 190 grams) when injected subcutaneously in amounts of 0.5 cc. at each twelve-hour interval. For the work tests the animals were anesthetized with phenobarbital sodium and the gastrocnemius muscle was loaded with 100 grams and made to contract three times each second by faradic stimulation. Each animal received 5 cc. of 0.8 per cent sodium chloride solution at the beginning of the stimulation and at each succeeding twelve-hour interval. A detailed description of the apparatus and methods used here has been presented (1). All of the animals were killed for necropsy by exsanguination.

EXPERIMENTS AND RESULTS. Two experiments were conducted. In the first experiment twenty normal rats were killed in order to obtain control data on the weights of the adrenal glands. Eighty rats were

made to work and ten rats were killed at the end of each of the following periods of work: six, twelve, eighteen, twenty-four, forty-eight, seventy-two, ninety-six and one hundred and twenty hours. The data on adrenal weights are summarized in figure 1. A small but unreliable increase in the average weight of the adrenals was noted in the group which had worked for six hours. In the twelve-hour group several of the values for the weights of adrenals were above the range for normal values and I accept this average as probably representing a true increase in the weights of the adrenal glands. In all of the groups which had worked for longer

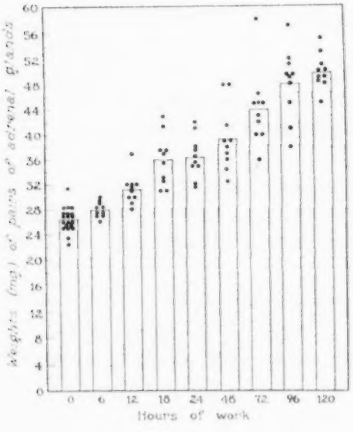

Fig. 1

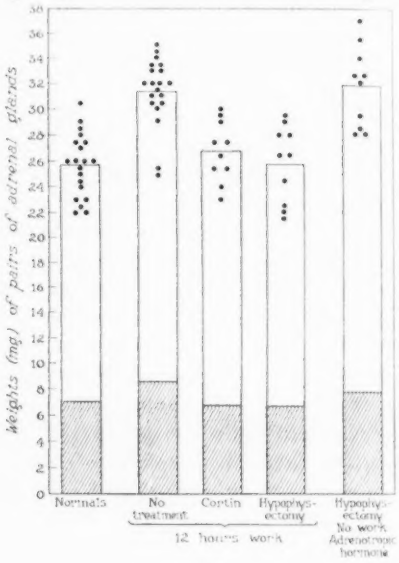

Fig. 2

Fig. 1. Effect of work on the weight of the adrenal glands of rats.

Fig. 2. Effects of work, treatment with cortin and hypophysectomy on the weight of the adrenal glands of rats. The average dry weights are shown by the shaded areas.

periods of time than twelve hours there was a progressive increase in the weights of the adrenal glands. The weights of the kidneys and the testes were also compared among the control and the worked animals in this experiment. No gains in weight above the range for normals were noted among the working animals.

In the second experiment the study was made in consideration of the hypothesis that the gains in weight of the adrenal glands represent a response to an increased requirement for cortin by the organism and that this response is mediated by the adrenotropic activity of the pituitary.

Twenty normal rats were killed in order to obtain additional control data. Twenty similar rats were made to work without treatment for twelve hours. Ten animals were treated with cortin in amounts of 1 cc. at the beginning of work and 0.5 cc. each hour for the first six hours of the twelve-hour work period. Ten animals were hypophysectomized and made to work until muscular responsiveness was lost. The range in time for exhaustion was from eight to eleven hours. Ten additional animals were hypophysectomized but were not made to work. Two cubic centimeters of adrenotropic hormone were injected subcutaneously into each of them immediately after operation and 1 cc. of this extract was injected at intervals of two, four, six and eight hours after operation. These animals were killed twelve hours after operation. Both wet and dry weights were obtained for the adrenal glands at necropsy.

The data of the second experiment are summarized in figure 2. Again there was a significant increase in the weights of the adrenal glands of the untreated animals which were made to work for twelve hours. This increase in weight did not occur in either those animals which were treated with cortin or those which were hypophysectomized, but not given adrenotropic hormone. Treatment of the hypophysectomized rat with large amounts of the adrenotropic hormone caused an increase in the weights of adrenals by twelve hours.

COMMENT. Significant gains in weight can be detected in the adrenal glands of rats as early as twelve hours after the beginning of stress. This increase in weight can be duplicated in the hypophysectomized rat by the injection of large amounts of the adrenotropic hormone. This gain in weight is not due entirely to an uptake of water by the adrenal tissues, since gains in dry weights were also noted. The weight response in the adrenal is probably an aspect of its functional adaptation to the increased requirement for cortin by the organism. It does not occur when cortin is injected frequently into the animal.

Selye and Collip have shown that chemical agents which ordinarily induce cortico-adrenal hyperplasia in rats do not have this effect in hypophysectomized animals, even when the adrenal cortices have been maintained at a normal size with pituitary extracts. Selye and Zwemer have each described cellular changes in the adrenal cortex occurring within a few hours after the beginning of a stress. It seems evident that functional adaptations may occur rather rapidly in the adrenal cortex and that they are mediated by the adrenotropic activity of the pituitary.

SUMMARY

When rats were anesthetized and the gastrocnemius muscle made to lift 100 grams three times per second a definite gain in the average weights of the adrenal glands was demonstrated to occur as early as twelve hours

after the beginning of work. This hypertrophy did not occur during work when similar rats were either hypophysectomized or treated with cortin. The administration of adrenotropic hormone to hypophysectomized rats likewise induced a hypertrophy of the adrenal glands by the end of twelve hours.

REFERENCES

(1) Heron, W. T., W. M. Hales and D. J. Ingle. This Journal **110**: 357, 1934.
(2) Selye, H. Brit. J. Exper. Path. **17**: 234, 1936.
(3) Selye, H. and J. B. Collip. Endocrinology **20**: 667, 1936.
(4) Zwemer, R. L. Am. J. Path. **12**: 107, 1936.

OBSERVATIONS ON THE pH OF THE ARTERIAL BLOOD, THE pH AND THE ELECTRICAL ACTIVITY OF THE CEREBRAL CORTEX[1]

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In this paper will be presented some results of experiments in which the pH of the arterial blood, the pH of the cerebral cortex and the electrical activity of the cortex were recorded simultaneously under various experimental conditions.

The techniques employed have been fully described in previous papers (1, 2, 3, 4, 5, 6). Specially constructed glass electrodes in conjunction with vacuum tube-microvoltmeters allow continuous photographic records of the pH of the blood and the cortex. The electrical activity of the cortex was recorded separately with Ag-$AgCl_2$ electrodes through AC-amplifiers and a Westinghouse four-element oscillograph.

With these techniques it can be shown (I) that under certain conditions changes in the pH of the cortex are parallel to those in the arterial blood; (II) that under other conditions changes in the pH of the cortex are not parallel to those of the arterial blood.

I. A striking instance of the first group of conditions can be obtained in experiments in which the artificial respiration is changed in the curarized animal. In such experiments a decrease of the artificial respiration promptly results in an acid shift in the pH of the blood and cortex. After restoration of the artificial respiration to the initial volume the pH of both blood and cortex rapidly returns to its original level. The changes in the pH of the cortex lag slightly behind those of the blood and are usually less rapid. Obviously under these conditions the pH of the cortex passively follows that of the arterial blood. See figure 1.

II. That the pH of the cortex need *not* follow the pH of the arterial blood can be inferred from observations in which the pH of one portion of the cortex is changed by local treatment which leaves the pH of the rest of the cortex unchanged. Local thermocoagulation (1, 2) or local electrical after-discharge following electrical stimulation (2) gives a local

[1] The expenses of this investigation were defrayed by a grant from Child Neurology Research (Friedsam Foundation) to J. G. D de B.

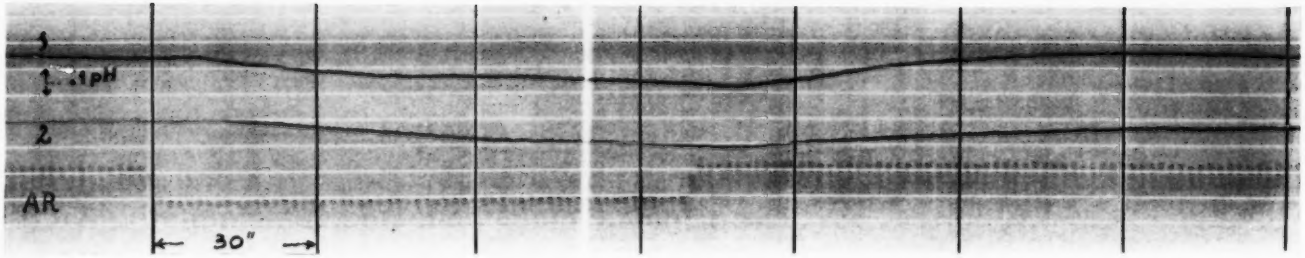

Fig. 1. Curarized dog under artificial respiration (*AR*). Record *1* = pH of arterial blood (femoral artery). Record *2* = pH of cerebral cortex. Note that both pH of blood and cortex shift towards acid side upon decrease of artificial respiration and return towards previous pH level upon restoration of original volume of the artificial respiration. Blood made non-coagulable by intravenous injection of chlorazol fast pink.

acid shift in the area involved; local freezing or local application of iodoacetic acid gives a local alkaline shift in the area involved. See figure 2.

Direct evidence that the pH of the cortex does not always parallel that of the arterial blood can be obtained in experiments with intravenous injection of monobromide of camphor.

Figure 3 shows the pH of the arterial blood and of the cortex and the simultaneous electrical activity of the cortex in a curarized dog under constant artificial respiration before and after an intravenous injection of camphor sufficiently large to produce a "central" epileptoid discharge of the cortex. It will be seen 1, that under these conditions the records

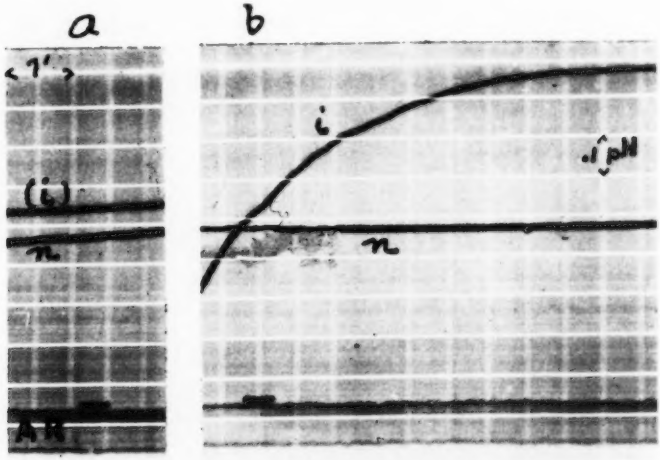

Fig. 2 shows the local development of alkalinity (record *i*) in an area of the cerebral cortex (monkey) treated with local application of iodoacetic acid (between sections a and b). pH of an adjacent untreated area remains unchanged (record *n*).

of the pH of the cortex and of the blood do not parallel each other; 2, that the record of the pH of the blood presents a steady slow acid shift, whereas the pH of the cortex, *ca* 80 seconds after the injection, shows a marked alkaline wave followed by a larger enduring acid wave and a subsequent return towards its original level at a time when the blood is still "going acid."

Figure 4 shows the pH records of the pre- and postcentral arm-areas and the electrical activity of these areas in a curarized monkey under constant artificial respiration following an intravenous injection of monobromide of camphor, which resulted in a "central" epileptoid discharge. It should be noted 1, that the changes in the two pH records although of

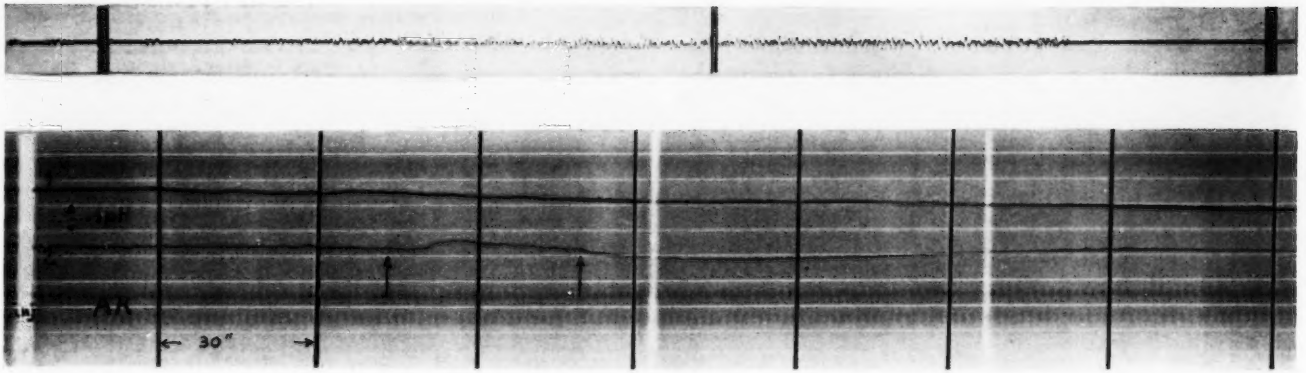

Fig. 3. The upper record shows an epileptoid electrical discharge in a (sensory) area of the cortex of a curarized dog following an intravenous injection of monobromide of camphor. The lower record shows the changes in pH of the blood (1) and of an area of the cortex (2) as close as convenient to that mentioned above. Note the alkaline wave followed by an acid swing in the record of the cortical pH and the non-parallelism of the two pH-curves. The period of the seizure is marked in the lower record by the two arrows. Time-markings in the two records coincide (30 sec.). *AR* = record of artificial respiration. Blood rendered non-coagulable by intravenous injection of chlorazol fast pink.

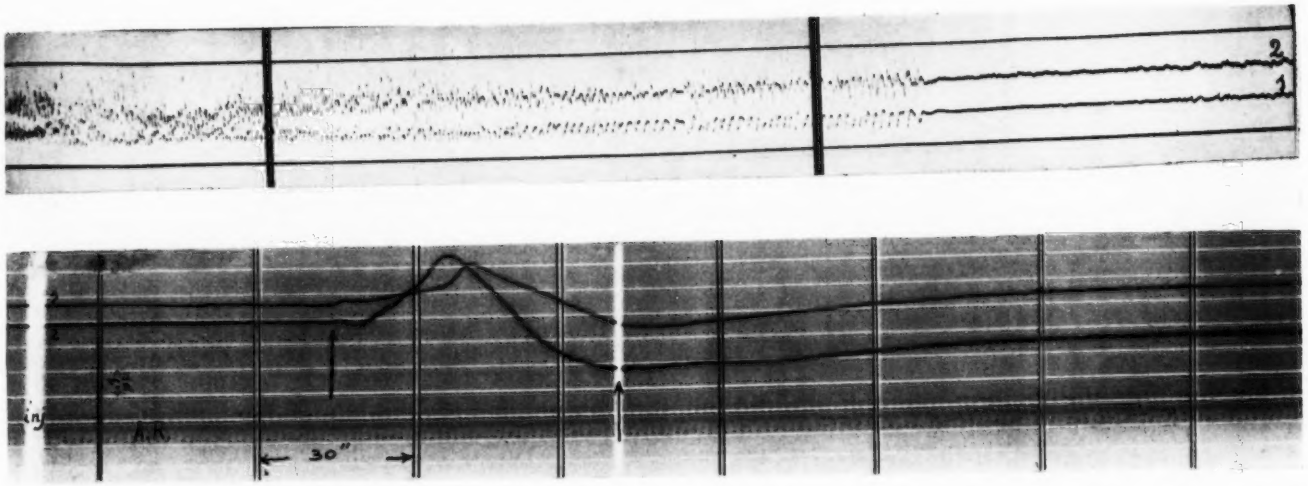

Fig. 4. The upper section shows the almost complete course (except for the very beginning) of a "central" epileptoid attack in a curarized monkey as reflected in the electrocorticograms of a pre- and postcentral area of the arm region, elicited by an intravenous injection of 1.2 mgm. of monobromide of camphor. *1* = record of electrocorticogram of A.4, *2* = record of electrocorticogram of A.1.2. The lower section of the figure shows the changes in pH of these same areas before, during and after the seizure. This began *ca* 55 seconds after the intravenous injection. The period of the seizure is indicated in the lower section of the figure by the two arrows. The time lines in the two records coincide (30 sec.). *AR* in lower section is record of the artificial respiration.

the same direction do not parallel each other exactly; 2, that the changes are much greater than those in figure 3, probably due to the fact that the epileptoid cortical discharge was much more severe in the experiment on the monkey.

From these records it seems permissible to conclude that the initial alkaline shift of the pH of the cortex is associated with the onset of the "central" epileptoid seizure and as the seizure proceeds the cortex becomes "acid."

Taken together these observations show that as soon as any alteration in the activity of the cortex occurs, its pH no longer passively reflects that of the arterial blood.

REFERENCES

(1) DUSSER DE BARENNE, J. G., W. S. MCCULLOCH AND L. F. NIMS. Proc. Soc. Exper. Biol. Med. **36**: 462, 1937.
(2) DUSSER DE BARENNE, J. G., W. S. MCCULLOCH AND L. F. NIMS. J. Cell. and Comp. Physiol. **10**: 277, 1937.
(3) NIMS, L. F. Yale J. Biol. Med. **10**: 241, 1938.
(4) NIMS, L. F., C. MARSHALL AND H. S. BURR. Science **87**: 197, 1938.
(5) NIMS, L. F. AND C. MARSHALL. Yale J. Biol. Med. **10**: 445, 1938.
(6) MARSHALL, C. AND L. F. NIMS. Yale J. Biol. Med. **10**: 561, 1938.

THE SYMPATHETIC NERVOUS SYSTEM AND ANAPHYLACTIC SHOCK

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Previous publications by one of us (Lissák and Kokas, 1935, 1936a) showed that in dogs in which the adrenal glands were ligated and the spleen denervated, anaphylactic and histamine shock produced contraction of the spleen. This contraction could not have been caused by asphyxia (bronchial constriction) or by a compensatory release of adrenine, but was rather due to an agent acting directly on the smooth muscle of the spleen. Further evidence that anaphylactic shock affects smooth muscle directly is offered by the fact that an isolated strip of spleen from a sensitized dog contracts when homologous protein is added; this contraction is followed by specific desensitization. Dale (1913) was the first to demonstrate that the typical reactions of anaphylactic shock can occur in denervated and isolated organs. Feldberg and his co-workers (Bartosch, Feldberg and Nagel, 1932, 1933) by perfusing the isolated lungs of sensitized guinea pigs with homologous protein brought about an anaphylactic bronchial constriction. Using the method of Langendorff to perfuse the isolated heart of sensitized guinea pigs with homologous protein Went and Lissák (1935a, b; 1936a) observed the anaphylactic reaction. Seastone and Rosenblueth (1933) recorded contraction of the totally denervated nictitating membrane of the cat during anaphylactic shock and thus brought additional support to the idea that contraction can occur without the mediation of autonomic nerve impulses. Besides these observations numerous similar experiments by others indicate that it is improbable that the autonomic nervous system has an important rôle in the phenomena of anaphylaxis. The opinion was expressed in a previous paper (Went and Lissák, 1936b) that in the course of anaphylactic shock biologically active substances of different nature are freed from the various tissues: e.g., histamine from the lungs, adrenine from the adrenal glands, and choline from the heart muscle. These substances might affect the corresponding organs in such a way as to produce the characteristic symptoms.

In order to investigate further whether the sympathetic nervous system

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plays any significant rôle in the phenomena of anaphylactic shock, we have carried out the present series of experiments on completely sympathectomized cats and also on sensitized normal cats under the influence of drugs which are known to modify the action of sympathetic nerves (933F, ergotoxine). As indicators of shock we have used the arterial blood pressure, the nictitating membrane and bronchial volume. In all the animals histamine was injected intravenously after specific desensitization, in order to compare histamine shock with shock otherwise produced and also to prove that shock can occur when other agents did not induce it.

METHOD. Cats weighing 2.5 to 3.5 kgm. were used. The technique of sympathectomy was that described by Cannon et al. (1929). The superior cervical ganglia and their postganglionic fibers were left intact in all but two cases; in them the superior cervical ganglion was removed on one side. For sensitization the animals received, first, 1 cc. of fresh dog serum intravenously; three days later the same amount of serum was injected. The experiments were carried out 20 to 25 days thereafter. The sympathectomized animals were anesthetized with urethane (1.25 gram per kgm., intravenously) and the normal cats with dial (Ciba, 0.6 to 0.8 cc. per kgm., intraperitoneally).

The arterial blood pressure was recorded from the right carotid artery (in some cases from the left femoral artery) by means of a mercury manometer. To record the contractions of the nictitating membrane, a small serrefine was placed on the outer margin and connected directly to a kymograph lever. The technique employed to detect alterations of the bronchial volume was that described by Went and Drinker (1929).

The serum and drugs were injected into the femoral vein. The drugs used were: histamine (ergamine, Burroughs-Wellcome), ergotoxine (Burroughs-Wellcome) and 933F (piperidinomethylbenzodioxane), kindly supplied by Dr. D. Bovet.

RESULTS. A. *Normal sensitized cats.* Five sensitized cats anesthetized with dial were injected with 0.5 cc. of fresh dog serum intravenously, and the change in arterial blood pressure, the movement of the nictitating membrane and the constriction of the bronchioles recorded. In two cases the left superior cervical ganglion had been removed two weeks previously, and in four cases the adrenals were ligated. The characteristic fall in blood pressure and bronchial constriction appeared 50 to 80 seconds after the injection of the dog serum. The nictitating membrane (relaxed under dial anesthesia) contracted only in the two cases in which the membrane was sensitized by previous removal of the superior cervical ganglion. One of these animals was the only case in which the adrenals had not been ligated. The contraction of the membrane was greater than in the animal in which the adrenals were tied. When histamine was injected the nictitating membrane contracted in all cases, and the blood-pressure and

bronchial-volume changes were similar to those produced by anaphylactic shock. A second injection of dog serum caused no renewed symptoms of shock, thus indicating desensitization.

B. *Sympathectomized sensitized cats.* Six sympathectomized and sensitized cats were anesthetized with urethane and injected with 0.5 cc. of

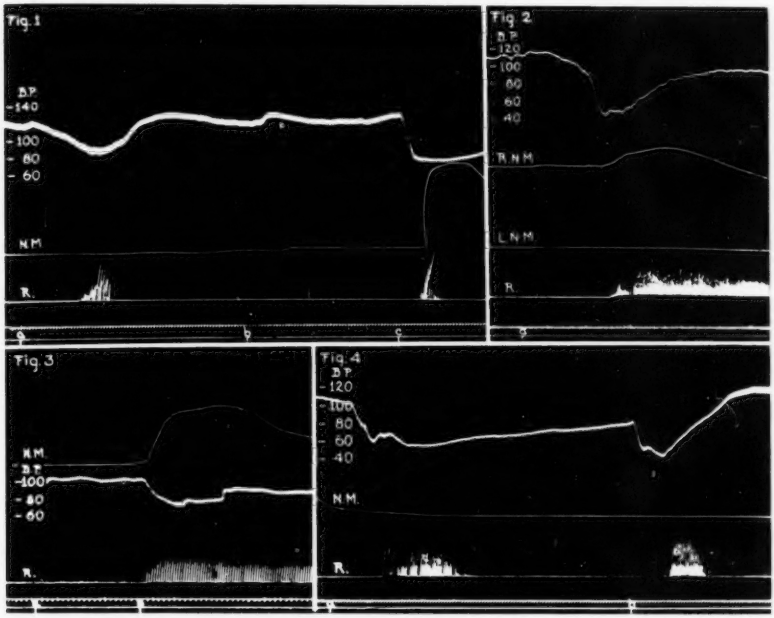

Fig. 1. Sympathectomized, sensitized cat. *BP*, arterial blood pressure; *NM*, nictitating membrane; *R*, bronchial volume. The time signal in this and in the following records represents five seconds. At *a*, 0.5 cc. fresh dog serum injected; at *b*, 0.8 cc. dog serum; at *c*, histamine (0.5 cc., 1:2,000).

Fig. 2. Sympathectomized, sensitized cat. Right nictitating membrane (*R.NM*) sensitized by previous removal of right superior cervical ganglion; left membrane (*L.NM*) normal. At *a*, 0.5 cc. dog serum injected.

Fig. 3. Normal sensitized cat injected with 933F (4 mgm. per kgm.). At *a*, 0.5 cc. dog serum injected; at *b*, histamine (0.5 cc., 1:2,000).

Fig. 4. Normal sensitized cat injected with ergotoxine (3 mgm. per kgm.). At *a*, 0.5 cc. dog serum; at *b*, histamine (0.5 cc., 1:2,000).

fresh dog serum. The symptoms of anaphylactic shock were not significantly different from those of the normal sensitized cats. Figures 1 and 2 illustrate the results. There was no difference in the fall of the arterial blood pressure and in the bronchial constriction. The nictitating membrane contracted only in those animals in which it was sensitized by

previous removal of the superior cervical ganglion. A second injection (0.8 cc. of dog serum) produced no response, proving that specific desensitization had occurred. Here again histamine caused a typical shock.

C. *Sensitized cats treated with 933F.* In four out of six normal sensitized cats the adrenals were ligated under dial anesthesia, and 933F (3 to 4 mgm. per kgm.) was injected intravenously. After 3 to 4 minutes, when the blood pressure was constant, 0.5 cc. of dog serum was injected. As figure 3 shows, after the injection of the homologous protein there was no sign of anaphylactic shock. In the other two animals the adrenal glands were not tied. The results were similarly negative. In contrast to this absence of anaphylactic shock, with histamine there was a prompt appearance of the characteristic effects (fig. 3).

D. *Sensitized cats treated with ergotoxine.* In four sensitized cats the adrenals were ligated under dial anesthesia and ergotoxine (3 mgm. per kgm.) injected. After 3 to 4 minutes, when the blood pressure became constant, the animals were given 0.5 cc. of dog serum. The usual anaphylactic shock symptoms appeared about 2 minutes after the injection (fig. 4a). The nictitating membrane, which was contracted after ergotoxine, relaxed slightly. The histamine shock was similar to the anaphylactic shock (fig. 4b). In three cases histamine also caused relaxation of the membrane.

Discussion. The experiments reported in sections A and B show that the removal of the sympathetic nervous system does not influence significantly the course of anaphylactic shock. It is interesting to note that even without the compensatory mechanism of both the sympathetic nervous system and the adrenals, anaphylactic shock is not fatal, and the animals may recover and be thrown into shock again by the addition of histamine. It is also apparent from the work of others (cf. Seastone and Rosenblueth, 1933) that the parasympathetic nervous system is not significant in the symptomatology of anaphylactic shock. In addition, atropine has been shown by Lissák and Kokas (1936b) to have no influence on the anaphylactic shock of dogs.

The following possibilities may be considered as explaining why anaphylactic shock is not observed after 933F (section C, fig. 3). *1*, 933F may produce some alteration in the state of sensitization. *2*, 933F may decrease cellular permeability (Rosenblueth and Cannon, 1936) so that the shock substances cannot enter or be liberated to produce their effect on other cells. Such a change in permeability, however, would probably not be an important factor in the disappearance of the shock symptoms since histamine still produces its typical action. *3*, 933F may promote the destruction of the humoral agents responsible for the shock, as it promotes that of adrenine and sympathin (Lissák, 1938).

The results with ergotoxine (section D, fig. 4) support the conclusion

which was drawn from the experiments on sympathectomized animals; namely, that the sympathetic nervous system has no significant influence on anaphylactic shock.

SUMMARY

1. The symptoms of anaphylactic shock are not significantly different in normal and completely sympathectomized cats (sections A and B, figs. 1 and 2).

2. The drug 933F prevents the appearance of anaphylactic shock in the cat, but does not influence histamine shock (section C, fig. 3). Possible mechanisms of this action are mentioned.

3. Ergotoxine has no effect on anaphylactic and histamine shock (section D, fig. 4).

REFERENCES

BARTOSCH, R., W. FELDBERG AND E. NAGEL. Pflüger's Arch. **230:** 647, 1932.
Ibid. **231:** 616, 1933.

CANNON, W. B., H. F. NEWTON, E. M. BRIGHT, V. MENKIN AND R. M. MOORE. This Journal **89:** 84, 1929.

DALE, H. H. J. Pharmacol. **4:** 167, 1913.

LISSÁK, K. This Journal **124:** 62, 1938.

LISSÁK, K. AND F. KOKAS. Arch. exper. Path. u. Pharmakol. **179:** 603, 1935.
Ibid. **182:** 504, 1936a.
Berichte ges. Physiol. **94:** 661, 1936b.

ROSENBLUETH, A. AND W. B. CANNON. This Journal **116:** 414, 1936.

SEASTONE, C. V., JR. AND A. ROSENBLUETH. J. Immunol. **27:** 57, 1933.

WENT, S. AND C. K. DRINKER. J. Exper. Med. **49:** 21, 1929.

WENT, S. AND K. LISSÁK. Arch. exper. Path. u. Pharmakol. **178:** 609, 1935a.
Ibid. **179:** 616, 1935b.
Ibid. **182:** 509, 1936a.
Magyar Orvosi Archivum **37:** 1, 1936b.

THE BROMSULPHALEIN LIVER FUNCTION TEST AS A METHOD OF ASSAY OF LIPOCAIC[1]

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In 1936, L. R. Dragstedt, Van Prohaska and Harms (Dragstedt, Van Prohaska and Harms, 1936; Van Prohaska, Dragstedt and Harms, 1936) reported that the beneficial effect of raw pancreas in preventing fatty infiltration of the liver in depancreatized dogs treated with insulin and in permitting these animals to survive, was due to its content of a new internal secretion which they called lipocaic. The active principle was secured in fat-free alcohol extracts of pancreas and some attempt was made to purify the product. The criteria indicating activity of a test fraction, adopted by these workers, were exceedingly laborious and time-consuming. It was first necessary to depancreatize several animals, place them on standard diets, and measure the daily glucose excretion and insulin requirement. After 4 to 6 weeks, when the clinical symptoms suggested the appearance of a fatty liver (decrease in glucose excretion, decrease in insulin tolerance, loss of appetite, and decreased activity), a laparotomy was done, the condition of the liver inspected, and a small portion removed for microscopic examination. If the presence of fat in the liver was confirmed by examination of the sections, the animals were then fed varying amounts of the fraction to be tested. The test was regarded as positive if the animals thereupon became more active, improvement in appetite occurred, glucose excretion markedly increased, and insulin tolerance returned to approximately the value found immediately after operation. In every case the conclusion as to the activity of a fraction was further verified by a third laparotomy, inspection of the liver, and microscopic examination of a small segment. Three depancreatized dogs and a minimum of 3 months were usually required for a single assay.

MacKay (1937) has recently reported that lipocaic preparations secured by the method of Dragstedt, Van Prohaska, and Harms are effective in

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preventing and relieving the fatty infiltration which occurs in the livers of normal rats that are placed on a high-fat low-protein diet and has suggested that this observation may provide a more rapid and convenient method of assay. The observations of MacKay were of great interest to us, but his method was not adopted, largely because we could not be sure that this metabolic defect in rats, where the pancreas was intact, was the same as that occurring in insulin-treated depancreatized dogs. Subsequent studies have verified this position, since both Aylward and Holt (1937) and Best and Ridout (1938) have reported that the lipotrophic effect of pancreas and of extracts of pancreas containing lipocaic on the fatty livers of rats can be accounted for on the basis of the choline and protein content. Dragstedt, Van Prohaska and Harms (1936) on the other hand proved that the beneficial effect of fresh pancreas or of their preparations of lipocaic on the fatty livers of depancreatized dogs was not due to choline or to pancreatic enzymes.

The almost complete replacement of the hepatic parenchyma by fat in the insulin-treated depancreatized dog suggested to us that varying degrees of liver insufficiency might be present in these animals, be detectable by appropriate methods, and serve as an additional criterion of lipocaic deficiency and thus as a convenient means for the assay of pancreas fractions. After a brief trial, the bromsulphalein test was selected. Two milligrams of the dye per kilo of body weight were usually injected and a blood sample secured at the end of 20 minutes. Tests on 14 normal dogs revealed complete disappearance of the dye from the serum in that period.

Over 400 bromsulphalein tests have been performed on some 60 depancreatized dogs treated with insulin. In general a retention of 10 to 20 per cent was observed for the first few days after pancreatectomy. It is probably significant that control of the diabetes with insulin during this early period was usually incomplete, as evidenced by the appearance of large amounts of sugar in the urine. The test then commonly disclosed a normal liver function for a varying period of 2 to 5 weeks, after which retention of the dye was again observed together with the clinical signs and symptoms of fatty liver enumerated above. In 22 cases a biopsy of the liver was performed from 1 to 4 days after the liver function test. In 17 of these the test showed a retention of from 10 to 30 per cent of the bromsulphalein and the corresponding liver biopsies contained from 12 to 42 per cent fat. In 3 cases the test showed a normal liver function and the biopsy specimens contained from 4 to 7 per cent total lipids, which is within the normal range. In the remaining 2 cases, the liver function tests were normal but the biopsies revealed the presence of a moderate degree of fatty infiltration. One of these animals died of a wound infection following biopsy, the other developed a more pronounced fatty infiltration of the liver and corresponding retention of bromsulphalein. In general,

a retention of bromsulphalein of from 10 to 20 per cent twenty minutes after injection was found to indicate a liver containing from 12 to 20 per cent total lipids (calculated as wet weight of the liver), and retention of from 20 to 30 per cent to indicate 20 to 40 per cent lipids.

The beneficial effect of lipocaic on the fatty liver of insulin-treated depancreatized dogs has also been demonstrated by the bromsulphalein test. In 7 such animals there was a significant dye retention and the presence of a fatty infiltration of the liver was further corroborated by laparotomy, inspection of the liver, and biopsy with chemical determination of the fat content. An adequate dose of lipocaic was then given daily in the food and in each case the liver function returned to normal or improved markedly in 10 to 16 days. Treatment with lipocaic was continued, the animals continued to improve, and after 2 to 4 weeks a second laparotomy with inspection and biopsy of the liver showed a decrease or complete disappearance of the fatty infiltration, thus confirming the conclusions derived from the bromsulphalein tests. It is significant that the liver function test reveals the beneficial action of lipocaic long before this effect can be definitely determined by the gross or microscopic appearance of the liver. It is necessary in most cases to wait 3 to 6 weeks when the histological changes become sufficiently marked to be conclusive. Improvement in liver function roughly parallels the improvement in clinical symptoms, increase in glucose excretion, and increase in insulin tolerance. In one of the experiments where liver function remained normal for several weeks under lipocaic therapy, retention of the dye reappeared within 10 days when the lipocaic was discontinued.

The persistence of bromsulphalein retention during the administration of a test material for 2 to 3 weeks indicates inactivity of the fraction. In seven such experiments, subsequent laparotomy, inspection of the liver and biopsy confirmed the conclusions derived from the liver function tests. The same pancreas fractions, proved to contain no lipocaic or at least an inadequate amount, were then given to 6 additional depancreatized animals with impaired liver function and again no improvement in liver function was produced. In each instance the bromsulphalein test accurately confirmed the known lack of potency of the test fraction.

A depancreatized dog was maintained on insulin and lipocaic for 80 days after operation without developing impaired liver function. Seventeen days after lipocaic was discontinued, bromsulphalein retention appeared and became progressively worse during the next 2 weeks. Lipocaic was then again supplied and the liver function became normal in 12 days.

The bromsulphalein test on depancreatized dogs as a means of assay for lipocaic is inaccurate if performed in the presence of infection, such as extensive subcutaneous or severe wound abscesses, and distemper. These infections produce impaired liver function and very severe fatty infiltra-

tion in such animals, which may be of different significance from that produced by lipocaic deficiency. The test is also probably unreliable if performed within a few days after ether anesthesia. Although ether anesthesia has been found to have no effect on the bromsulphalein excretion of normal dogs (C. A. Dragstedt and Mills, 1936), it produces a definite retention in some depancreatized animals for 2 to 3 days.

COMMENT. The frequent occurrence of impaired liver function in diabetes mellitus has been pointed out by Meyer (1931), in whose series of diabetics 7 per cent showed both clinical and laboratory evidence of hepatic dysfunction and 28 per cent evidence of decreased liver function as revealed by the bromsulphalein test. It seems probable that a considerable proportion of these cases are due to fatty infiltration in the liver and of these a proportion may be due to lipocaic deficiency. Snell and Comfort (1937) have also called attention to the occurrence of liver damage in patients with diabetes, particularly where there has been evidence of extensive injury to the pancreas. In one such patient with enlargement of the liver, ascites, and decreased bromsulphalein excretion, the administration of lipocaic produced improvement in both symptoms and liver function. Grayzel and Radwin (1938) treated three young diabetics with hepatomegaly by means of lipocaic and secured a striking recession of the liver to its normal size. When the lipocaic was discontinued, the hepatomegaly returned and again receded when lipocaic was resumed. In these patients the hepatomegaly was not due to poor control of the diabetes by inadequate administration of insulin. David H. Rosenberg (1938) treated a case of hepatomegaly with fatty metamorphosis of the liver in an adult with lipocaic and secured a complete return of the liver to its normal state. This case is particularly valuable since a laparotomy previous to treatment made it possible to inspect the liver and secure a biopsy specimen and a second laparotomy after the treatment with lipocaic made it possible to confirm the impression of recession of the liver and improvement in function as evidenced by liver function tests.

CONCLUSIONS

1. Impaired liver function as evidenced by abnormal retention of bromsulphalein has been found in the large majority of insulin-treated depancreatized dogs that develop fatty infiltration of the liver.

2. The disturbance in liver function has been found to be roughly proportionate to the amount of fatty infiltration and to respond to lipocaic therapy more rapidly. Accordingly the bromsulphalein test is suggested as an additional criterion of lipocaic deficiency in depancreatized dogs and as an aid in the assay of pancreas fractions for this substance.

3. The employment of the bromsulphalein test as an indicator of lipocaic deficiency in diabetes mellitus is discussed.

REFERENCES

(1) Aylward, F. X. and L. E. Holt. J. Biol. Chem. **121:** 61, 1937.
(2) Best, C. H. and J. H. Ridout. This Journal **122:** 67, 1938.
(3) Dragstedt, C. A. and M. A. Mills. Proc. Soc. Exper. Biol. Med. **34:** 467, 1936.
(4) Dragstedt, L. R., J. Van Prohaska and H. P. Harms. This Journal **117:** 175, 1936.
(5) Grayzel, H. G. and L. S. Radwin. Am. J. Dis. Children **56:** 22, 1938.
(6) MacKay, E. M. This Journal **119:** 783, 1937.
(7) Meyer, E. L. Arch. Int. Med. **47:** 182, 1931.
(8) Rosenberg, D. H. Meeting of the American Gastro-Enterological Association, Atlantic City, May 2 and 3, 1938.
(9) Snell, A. M. and M. W. Comfort. Am. J. Digest. Dis. and Nutrition **4:** 215, 1937.
(10) Van Prohaska, J., L. R. Dragstedt and H. P. Harms. This Journal **117:** 166, 1936.

VASCULAR EVENTS AND VOLUME CHANGES IN THE TURTLE'S LIVER SURVIVING IN AN ONCOMETER

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Experiments with the surviving turtle's liver have shown that small, short-lasting changes in the physical status or the chemical composition of the perfusing fluid may bring about profound changes in the minute-volume outflow from that organ (for literature see references in Snyder and Johnson, 1938). This suggests that the volume of the liver also, under similar conditions may undergo similar changes. In order to observe such changes a suitable oncometer finally has been devised together with a recording plethysmograph and a method of isolating one lobe of the turtle's liver and transferring it into the chamber without serious interruption of its portal-hepatic venous circulation.

The transference to the oncometer having been accomplished and the optimal conditions having been ascertained and established, the perfusion is maintained at constant conditions long enough to permit an equilibrium to be set up between perfusing fluid and tissue-cells. Records are then taken on a smoked drum driven by a synchronous electric motor.

Control experiments lasting from thirty minutes to an hour were carried out as well as experiments during which for short periods of time drugs, known to affect the liver functions, were injected.

The perfusion fluid. It being assumed at the outset that a fluid suitable for the surviving, small intestine also may be more suitable for surviving liver than a simpler saline solution, Tyrode's was chosen as a basis for the perfusing solution. This was modified by adding 1.5 per cent gum acacia (gum arabic, white no. 1, U.S.P.). The acacia was used according to the experience of Erlanger and Gasser (1919) and proved very efficacious in preventing edema and loss of protein from the liver-cells. After chilling the solution in the cold room a stream of gas-mixture of $97\frac{1}{2}$ per cent oxygen and $2\frac{1}{2}$ per cent carbon dioxide was passed through to saturation. The pH level was finally adjusted by adding more CO_2 or washing out its excess with pure oxygen. The containers were sterilized in the autoclave before using and dextrose (Pfanstiehl's d-glucose) was added only just before the experiment was begun.

The oxygen supply. No experiments have been carried out as yet to determine the normal consumption of oxygen by surviving turtle's liver.

A review of the literature permits a rough estimate of what one may expect to find: According to Burton-Opitz (1915) the combined arterial and portal blood at 37°C. flowing though the dog's liver amounts to *ca* 84 ml. per 100 grams liver per minute. Extrapolating from this for the liver of the cold-blooded animal we may say tentatively that 100 grams turtle's liver at 17°C. would require a rate of flow of *ca* 14 ml. of blood per minute From the data of Blalock and Mason (1936) on the oxygen consumption by dog's liver and that of Lundsgaard (1938) on the oxygen consumption by cat's liver, extrapolation for the cold-blooded animal at 18°C. leads to 0.72 ml. O_2 and 0.50 ml. O_2 respectively for 100 grams dog's and cat's liver per minute. Taking the smaller figure for 100 grams turtle's liver at 18°C., and the figure for physical solution of O_2 in water at the same temperature, 3 ml./100 grams, the rate of flow through 100 grams of liver should be 0.50/0.03, or 17 ml. of perfusion fluid per minute. The weights of the right lobes of the 16 preparations used varied between 27.4 and 53.5 grams, whose mean is 39.5 grams. The mean rate of flow through turtle's liver preparation weighing 40 grams may therefore be about 0.4×17, or 6.8 ml. per minute, if physiological saline is used, and 14×0.4, or 5.6 ml. per minute if full blood is used.

The rate of flow through the preparations here reported upon was usually slower than this estimated rate. The tissues therefore may have suffered anoxia at times. But it is quite likely that under normal conditions the rate of circulating blood through the liver of any animal from time to time varies greatly, what with fasting and feasting, activity and rest. Furthermore small deviations from the normal state, as every clinician knows, may also greatly affect the size of the liver. (Compare E, Forsgen, 1935.) Therefore until we know more definitely the oxygen requirement of the liver under varying conditions and the effects of prolonged anoxia we must hold conclusions in abeyance.

The construction of the liver oncometer. So far as the author knows, this is the first time the surviving turtle's liver has been studied in an oncometer. Several authors, however, have reported successful enclosure of the mammalian liver in oncometers. Lampe (1926) describes one such and, together with his co-workers, has published results of much research with this preparation on the livers of dogs, cats and rabbits, from the results of which they claim, among other things, that adrenalin produces vascular constriction in portal and hepatic venules and also in hepatic arterioles with a sphincter-like effect and always accompanied by diminution of liver-volume. They find also that physostigmin causes decrease of liver-volume in cats and dogs (Lampe and Mehes, 1927). Mautner and Pick (1930) studied the dog's liver enclosed within an oncometer. Lamson and Roco (1921) observed changes in liver size in the dog by x-ray shadows.

From the descriptions given of oncometers for mammalian livers one gets the impression that they are unwieldly and beset with great difficulties

in manipulation, compared to which the one described below for the cold blooded organ is in many ways to be preferred.

The form of chamber selected for the turtle's liver is a wide-mouthed one pint glass jar used for preserving food-stuffs and easily obtained in shops. It is known as the "Mason preserve jar." The jar is already provided with a glass cover, rubber gasket and heavy wire bails so shaped and attached that by a very simple manipulation the cover can be clamped on the jar in an air- and water-tight seal. To adapt this for an oncometer-chamber the glass cover was discarded and another of similar shape and exact size was turned out on the lathe from a plate of hard rubber. Holes of suitable size and at suitable positions were bored through this cover. Two pairs of glass tubes, all a few centimeters long and 6 mm. in diameter, were then sealed in the holes. One pair of the tubes is used for inlet and outlet for perfusion fluid and air to the extra hepatic space of the chamber and to the recording plethysmograph; the other pair is used as inlet and outlet between reservoir of perfusion fluid and the liver blood vessels. The original rubber gasket is used when sealing in.

To support the liver preparation a light stage is attached at right angles to the inside surface of the cover so that it extends into the whole length of the jar. This stage consists of a sheet of cotton gauze loosely stretched over and secured to a stainless steel wire-frame (attached to the inner surface of the cover), or a thin sheet of cork, which affords a bed for the preparation to lie on and a support for the preparation while making the connections and upon which the cannulae may be secured by threads or crossed pins. The preparation is held further in position on the bed by a loose jacket of cotton gauze. In making the transfer of the preparation from the animal's body to the oncometer the perfusion is interrupted only long enough to make the final connections between cannulae (already in their proper veins) and the oncometer outlet and inlet tubes. Practice reduces this delay to about 30 seconds. If inspection indicates there is no leak, the preparation is placed in the oncometer chamber; the latter is filled with fluid; the cover is sealed tightly and additional fluid is allowed to flow into the extrahepatic space to wash out the last air bubble. The chamber is then mounted securely in a special frame which holds the preparation nearly in the same position it occupies while in the supine body of the animal, and centrally in the chamber so that it is bathed on all sides by the extrahepatic fluid.

The connection with the recording end of the plethysmograph is now made and the inflow pressure as well as extrahepatic pressure adjusted to insure an optimal rate of perfusion. After all adjustments are completed the perfusion is allowed to continue undisturbed for 20 to 30 minutes longer to insure a steady state before the recording of the liver's performance is begun.

For measuring and recording the rate of outflow from the liver an automatically tilting vessel with electric contacts and electromagnetic recorder was constructed. The capacity at the "tilting-point" of this vessel can be varied (and set by thumb screw adjustment) to volumes varying from 5 to 15 ml. The recorder thus indicates at each signal on the drum a volume that is roughly constant. But to ensure a more accurate reading, the volume unloaded each time is received by a glass funnel whose stem extends into a graduated centrifuge tube of 15 ml. capacity. During the collections the volumes are at once read to the tenth ml. and noted in the protocols. From the recorded volumes and the corresponding time-intervals as they appear on the drum records we can calculate the minute volume outflow from the liver from time to time during the whole period of an experiment.

Method of isolating the liver preparation and sealing in oncometer. The right lobe of the liver of a turtle is isolated with inflow cannula inserted in the portal vein, ouflow cannula in the hepatic vein. To insure against leaks three ligatures of heavy thread, tying off *en masse a*, all the abdominal connections; *b*, all the connection with right lung; and, after the blood of the liver is all washed out by the perfusing fluid, *c*, the left liver lobe and the venous sinus to the left of the entrance of the large hepatic vein. All tissue distal (liver being proximal) to the three large ligatures is then cut away and the preparation may be lifted out of the body-cavity. If allowed to lie on a dry plate for a while any leaks will be detected; for, by intention, all the perfusing fluid should leave the preparation only by way of the outflow cannula. The gall bladder is left intact in the preparation; in the present experiments the common duct was always tied off. (By inserting the inflow cannula into the left ramus of the portal vein where it crosses the isthmus, and the outflow cannula into the hepatic vein from the left lobe, one would have a left lobe-preparation and no gall bladder with its wall of smooth muscle. However the abnormal state of obstructed gall ducts would still not be obviated.)

If the perfusion through the preparation appears to be satisfactory one transfers it to the oncometer and seals it into position, precautions being taken not to subject it to an excessive head of perfusion pressure and to clear the liver chamber and plethysmographic system of all air bubbles.

The head of perfusion pressure is then adjusted (10 to 15 cm. water column usually) to give a desired rate of flow. The optimal extrahepatic pressure in the liver chamber is found to be from 0 to 2 cm. of a water column; the higher this pressure, it is found, the higher the perfusion pressure must be to insure adequate perfusion rate. The mouth of the outflow tube from the hepatic vein is set at a level with inflow cannula at the portal vein, that is, referred to the mean hepatic capillary bed, at about zero pressure. While the preparation is coming into equilibrium with the "steady state" thus established (at least a half-hour is required)

the recording devices are adjusted for smooth performance covering the duration of the experiment.

Gain and loss in the liver's output. In the measurement of the liver's uptake and output of substances differences of concentrations per unit volume at the inflow and outflow often have been considered decisive. But as was shown in earlier papers and as appears in the sequel (Snyder, Johnson and Peek, 1938) knowledge of concentrations alone is not enough. For minute volume output, even though concentrations remain constant, may affect significantly the actual output of a substance when compared to the actual input of the same substance during equal and identical periods of time. The difference indeed must become enormous, at a time when minute volume outflow is diminishing while minute volume inflow is increasing, or *vice versa*. To get a truer knowledge of the liver's uptake and output of substances experimenters have analyzed portions of the liver itself, "before and after," for its content in water, protein and the particular substances they happened to be interested in. But this method is not feasible for the determination of uptake and output covering frequent and successive short periods of time.

Rate of inflow to the liver. Thus far we have not been able to devise a satisfactory method of measuring directly inflow volume to the isolated liver preparation covering successive, short periods of time. An indirect method however has been adopted which has obvious objections and therefore requires some detailed description and discussion:

As long as the plethysmograph shows no volume change it may be assumed that the volume inflow to the organ is equal to the volume outflow. But an increase or decrease of liver volume recorded by the plethysmograph may be the result of the inflow to the liver being greater or less than the outflow. In this case the volume inflow will be simply the algebraic sum of the volume outflow from the organ and the gain or loss in organ volume during the same period of time, and for an organ as vascular as the liver this no doubt is often the case. But the liver cells no doubt often take up and give off fluid. In other words, when the liver volume changes the change may be due to changes in the vascular bed or in the volume of the liver cells themselves, or both.

Filling and emptying of hepatic sinuses can be no certain index of substance transfer from or to liver cells. It is possible that there are mass condensations, and expansions going on within the liver cells resulting in volume changes that would mask the real volumes with which we are here concerned. Again to know fully from volumetric measurements the uptake or output of the cells of an organ, one would needs measure separately not only total organ- and blood-vascular volumes but also tissue-space and lymphatic vascular volumes. Then perhaps the final cell volumes could be had as a remainder.

The following method for the determination of inflow volumes of our

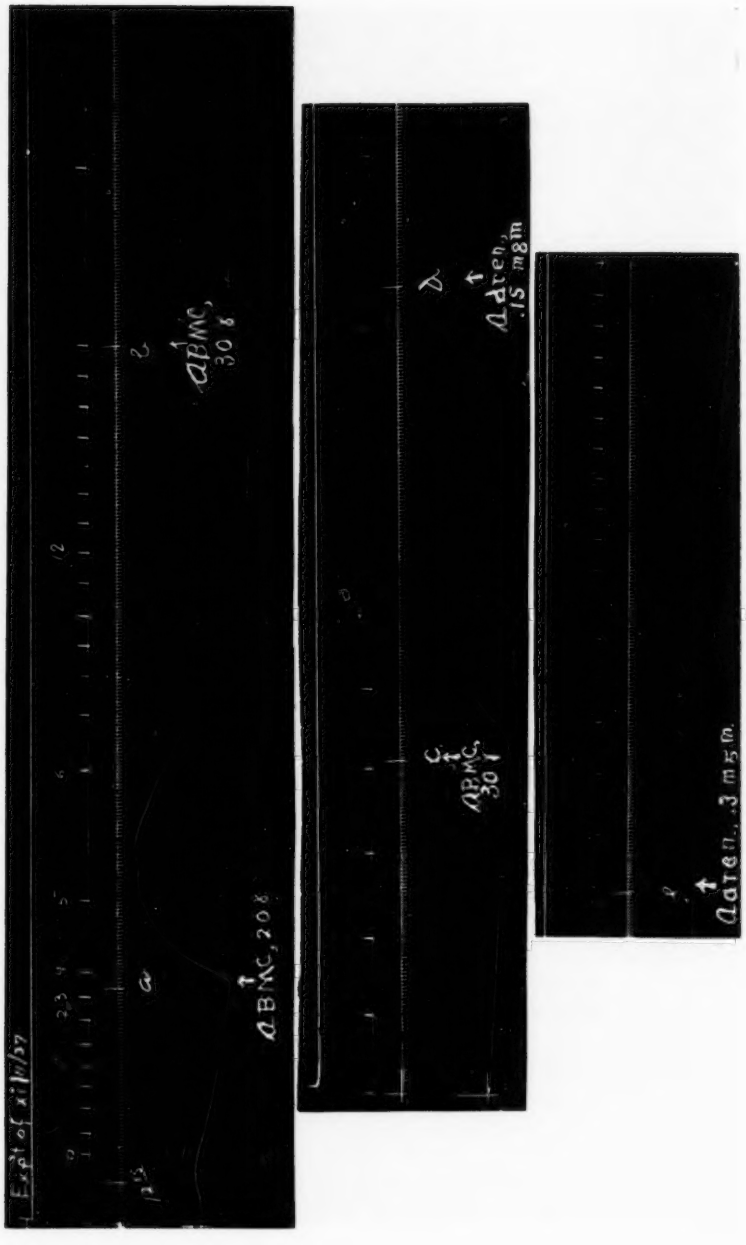
Expt of xii/57
ABMC, 20 8
ABMC, 30 8
Adren, .15 mgm
ABMC, 30 1
Adren, .3 mgm.

FIG. 1

preparation, therefore while not free from objections, is nevertheless adopted until a better method can be found. It will afford also a basis for the approximate estimation of uptake and output of substances by our organ as a whole for successive, short periods of time and will be used as such in a forthcoming publication (Snyder, Johnson, and Peek, 1938). The argument for the method as a measure of inflow volume is briefly as follows:

The volume outflow during the collection of a sample is put down as O_v, the synchronous change in liver volume is put down as dL_v. If $dL_v = 0$ it is inferred that the corresponding inflow volume (I_v) must have been just equal to the outflow volume. In general, $I_v = O_v + dL_v$, so that if there is gain or loss in either outflow volume or in liver volume during the collection of any subsequent sample the total gain or loss of outflow over inflow will be expressed by the equation, $O_v - I_v = -dL_v$.

RESULTS OF THE EXPERIMENTS. Examining now the experimental results, illustrated by figures 1 and 2 and tables 1 and 2, the following points may be stressed:

1. As the drum-record of figure 1 and the figures in column 4 of table 1 indicate, there is a general tendency, during the experiment, for the liver preparation to increase its volume. This is at the rate of *ca* 0.8 per cent per minute and is probably due to the low pH (= 7.2) of the perfusion solution. Small doses of adren.-HCl counteract this tendency to volume increase.

Fig. 1. The smoked drum record of the experiment of 11/11/37 in three sections, reduced to ½ of the original size. The four trace-lines in each section are from above downward: first, the inflow pressure tracing, 12.8 cm. water column, kept constant throughout; second, the outflow recording trace, each signal mark representing *ca* 8.7 ml.; fourth, the plethysmograph, recording liver-volume changes, upward movement indicating decrease, downward movement indicating increase of liver volume; third, chronograph-signal-key trace, marking time in intervals of 9.5 seconds each, upstroke of this tracing signals injection by needle syringe piercing the rubber tubing at the inflow. At the signals, *a*, *b* and *c*, AβMC in 0.01 per cent sol. was injected in doses of 20γ, 30γ and 30γ respectively; at *d* and *e* Adren.-HCl in 1:1000 concentration was injected, 0.15 mgm. and 0.30 mgm. respectively.

It will be noted that there is a tendency for the liver volume to increase throughout the record, the AβMC causing decrease of volume the duration of which increases with the dose. The rate of outflow from the liver is markedly decreased by AβMC, as had already been shown by Snyder (1936), and increased by the Adren.-HCl. The Adren.-HCl also counteracts the tendency to volume-increase of the liver without diminishing outflow. The effects of AβMC and Adren.-HCl on rate of outflow and liver volume appear also in the drum record reproduced in figure 2, II-a and II-b. The wet weight of the liver, free from gall bladder and all non-liver substance and after draining thoroughly of fluids (at the end of the expt.) was 40 grams. The pH of the perfusion fluid was 7.2, which probably accounts for the general tendency for vascular dilatation.

2. Acetyl-β-methylcholine (AβMC) causes not only marked decrease in rate of outflow (as shown previously, 1938) but also marked decrease

TABLE 1

	TREATMENT AND SAMPLE NUMBER		OBSERVED HEPATIC OUTFLOW		OBSERVED LIVER-VOLUME GAIN (+) OR LOSS (−)*	CALCULATED PORTAL INFLOW-VOLUME PER SAMPLE	CALCULATED MEAN GAIN (+) OR LOSS (−) OF OUTFLOW OVER INFLOW‡
			Time to collect	Volume collected			
			min.	*ml.*	*ml.*	*ml.*	*per cent*
Part I	AβMC	1	0.98	8.4	+0.4	8.8	−4.8
		2	1.13	8.8	+0.3	9.1	−3.4
		3	1.03	8.6	+0.2	8.8	− 2.3
		4	1.15	8.5	−1.8	6.7	+21.2
		5	3.30	8.5	−4.9	3.6	+57.7
		6	5.92	8.8	+1.9	10.7	−21.4
		7	2.52	8.6	+2.3	10.9	−26.7
		8	1.83	8.6	+1.4	10.0	−16.5
		9	1.43	8.5	+0.9	9.4	−10.6
		10	1.45	8.5	+0.8	9.3	−9.4
		11	1.55	9.0	+0.8	9.8	−9.5
		12	1.47	8.5	+0.6	9.1	−7.1
Mean				8.7			
Part II	AβMC	1	3.47	†	+0.3	9.0	−3.2
		2	3.93		+0.4	9.1	−4.5
		3	3.90		+0.4	9.1	−4.5
		4	3.69		−2.9	5.8	+33.6
		5	16.75		+3.6	12.3	−42.6
	Adren.	6	7.98		+0.9	9.5	−10.3
		7	4.55		0.0	8.7	0.0
	Adren.	8	3.11		0.0	8.7	0.0
		9	2.85		0.0	8.7	0.0
		10	2.39		+0.1	8.8	−1.1
		11	2.07		−0.1	8.6	+1.2
		12	1.81		+0.5	9.2	−5.9
		13	1.54		+0.5	9.2	−5.9
		14	1.54		+0.5	9.2	−5.9
		15	1.38		+0.3	9.0	−3.5

* These figures give the absolute increase or decrease in liver volume that is reached by the time the sample is collected and as can be read off from the calibration chart of the corresponding plethysmographic excursion.

† The mean value, 8.7, of the samples above this point is taken as the volume for the rest of the samples, these last not having been observed directly.

‡ The figures in this column, granting the validity of the method of determining the inflow volume (as described in the text and as given in column 5) give the percental gain or loss of outflow over inflow volume per sample collected.

in liver volume. Lampe and Mehes (1927) found that physostigmin constricts liver volume in cats and dogs.

3. This decrease in liver volume (see columns 5 and 6 of table 1) appears to be due to a decreased rate of inflow which is greater than the decrease

TABLE 2

A summary of chief data and maximum effects of AβMC and Adren.-HCl

DRUG USED	DATE OF EXPERIMENT	PERFUSING SOLUTIONS			AMOUNT OF DRUG INJECTED	VOLUME OF LIVER TISSUE* PERFUSED	MAXIMUM EFFECT OF DRUG‡ INJECTED		
			Pressure levels in cm. of H_2O					On liver volumes:	
		pH	At inflow cannula	Extra-hepatic space			On min. vol. outflow	Change in	Change as per cent of total volume
					γ	*ml.*		*ml.*	
AβMC	6/10/38	7.3	[12.0]	[1.0]	(1) 15	32	+15	−1.53	−4.8
					(2) 15		+44	0.00	0.0†
	3/24/38	7.55	12.5	1.0	20	32	−55	−1.24	−3.9
	2/25/38	7.55	11.0	1.0	30	52	−55	−3.10	−6.0
	2/10/38	7.2	12.0	3.0	25	36	−43	−7.8	−21.7
	1/15/38	7.3	13.0	1.0	25	26	−13	−3.49	−13.4
	12/17/37	[7.6]	18.5	5.6	20	43	−48	−0.01	−0.0
	12/ 4/37	7.55	18.7	4.5	33	39	−81	−5.50	−14.1
	11/18/37	6.9	15.5	1.0	35	50	−52	−3.00	−6.0
	11/11/37	7.2	12.8	0.5	20	38	−83	−4.60	−12.1
	11/ 5/37	7.2	13.0	2.5	20	[38]	−51	−0.40	[−1.0]
Adren.-HCl	6/ 3/38	7.3	[12.0]	[1.0]	(1)150	29	+69	None	0.0†
					(2)150		−18	−0.60	−2.1
	5/ 3/38	7.3	[12.0]	[1.0]	150	26	+826	+4.28	+16.5
	4/14/38	7.45	[12.0]	[1.0]	0	39	−21	0.00	0.0†
	2/10/38	7.2	12.0	3.0	350	35	+26	−6.50	−18.6
	2/ 4/38	6.9	13.0	1.2	200	51	+16	−2.00	−4.0
	1/15/38	7.3	13.0	1.0	200	28	+95	[−1.27]	[−4.5]
	12/17/37	[7.55]	18.5	5.6	200	43	+140	+4.00	+9.3
	12/ 4/37	7.55	18.7	4.5	200	39	+90	−8.90	−22.8
	11/18/37	6.9	15.5	1.0	300	50	(?)	−0.66	−1.3
	10/15/37	[7.6]	13.0	5.7	0	22	+34	0.00	0.0†
	10/29/37	7.6	15.0	3.8	250	37	+10	−1.21	−3.3

* The volume is that weight of the liver left after removing gall bladder, connective tissues and ligatures and after draining (at the end of the experiment) divided by 1.06, the assumed sp. gr.

† The volume is kept constant in two of these cases by clamping off the path to plethysmograph recorder; in the other two it will be noted no drug was given at all.

‡ The change in the minute-volume here recorded is the gain or loss over the mean values before and after the drug action took place.

Note. The figures in brackets are approximations only, the exact data being lacking in one respect or another.

in the rate of outflow (when at its maximum) by nearly 58 per cent. This appears to be accomplished by a vasoconstricting effect of the AβMC

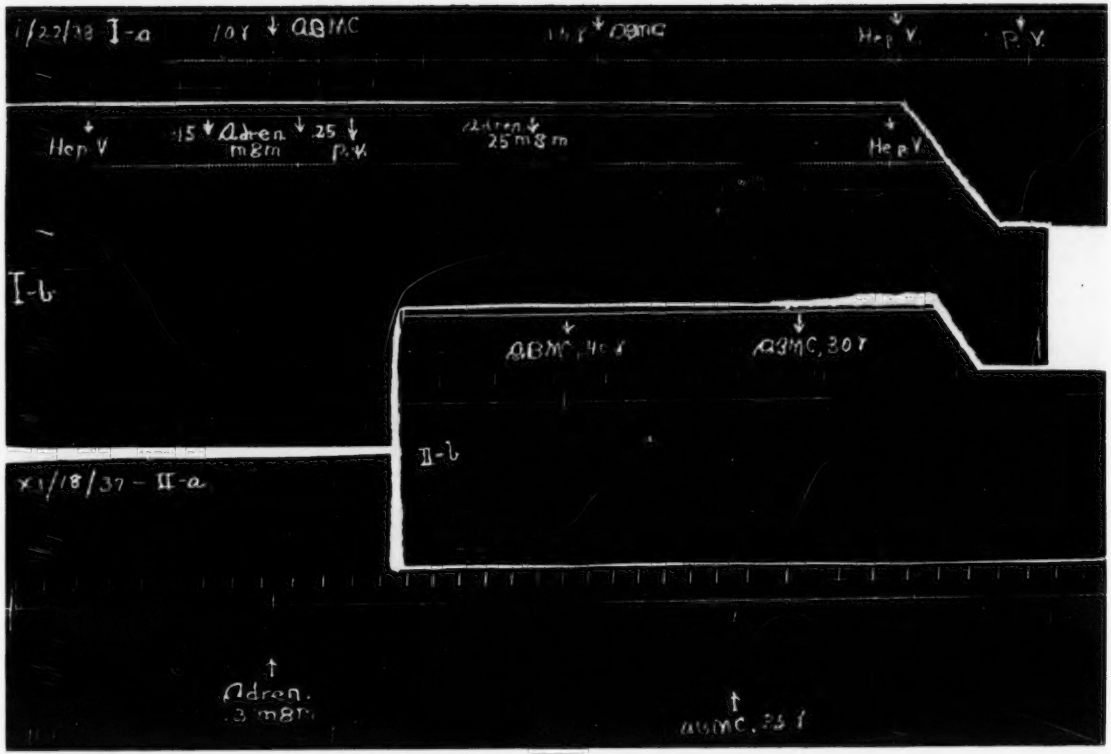
1/22/38 I-a
10 ↓ ABMC
15 ↓ ABMC
Hep V.
P. V.
Hep V
15 ↓ Adren .1 m8m
25 ↓ P. V.
Adren ↓ 25 m8m
Hep V
I-b
ABMC, 40 ↓
ABMC, 30 ↓
II-b
x1/18/37 - II-a
Adren. .3 m8m
ABMC, 25 ↓

Fig. 2

upon the portal veins. Inspection of the bubbling rate through the Mariotte tube of the reservoir convinced me that the inflow was completely shut off by the constriction at times for many minutes.

4. The absolute loss in liver volume at the maximum (tabulated) effect is 13 per cent of the volume of the liver substance perfused at start, which was (40.1 grams by weight, sp. gr. taken at 1.06) *ca* 38 ml.

5. But following the immediate great decrease in liver volume, due to AβMC injection, is a gradual increase of volume together with a reversal of the outflow-inflow-rate ratio. It appears that the expanding portal venous bed allows fluid to enter faster than it can pass through the capillary bed and out of the hepatic vein.

6. This rebound as an expansion of portal veins (presumably following the disappearance of the choline drug) may be promptly cut short by epinephrin, and the liver volume instead of increasing may be held at least at a somewhat steady level.

7. But in general the effect of adren.-HCl on the rate of liver outflow is augmentatory; of the AβMC, depressing. (See the summary of experimental results in table 2.) These contrary effects are doubtless due on the one hand to the dilating action of the epinephrin drug on the hepatic veins, on the other hand to the constricting action of the choline drug in the portal veins. Bauer, Dale and Richards (1929) observed that epinephrin relieved obstruction to the hepatic outflow in the dog, brought on by histamine, and inferred that the epinephrin had this effect by virtue of its dilating effect on the hepatic veins. Along with these specific

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Fig. 2. The drum records of two different experiments, reduced to ½ of the original sizes. The interpretation of the trace-lines is the same as for figure 1. II-a and b are the records of the experiment of 11/18/37 showing the effects of AβMC and Adren.-HCl on liver volume and rate of outflow of the perfusion fluid, the head of pres. of which was 15.5 cm. and the pH = 6.9. The weight of the active liver tissue alone was 52.5 grams. The samples taken for analyses were nos. 6–17 incl. and 24–35 incl. as they appear in order (left to right) in II-a of this figure, and on the rate-of-outflow tracing.

The I-a and I-b parts of this figure are from the experiment of 1/22/38, and show the behavior of the liver preparation to attempts at reversing the circulation suddenly (note the *Hep. V.* signals). As will be noted no fluid passed through in the direction, hepatic vein to portal vein; but, the head of pressure being kept the same, the volume of the liver is enormously increased. Upon turning the perfusing fluid back through the normal portal vein path (note the *P.v.* signals) both the flow through is resumed immediately and the enlarged liver shrinks gradually to its original volume. The small doses of AβMC injected (10γ and 15γ respectively) during the first part of the record, I-a, were given to test out the vascular responsiveness of the preparation. Adren.-HCl was injected during the reversed circulation, without avail. It will be noted that the head of pres. remains unchanged; it was set at *ca* 11.7 cm. of water. The conditions seem to be favorable for the liver to maintain nearly constant volume so long as no drug is added to the perfusate.

actions of these drugs on the vascular walls it is well to recall that some of the excess fluid in the outflow of the liver under epinephrin may be due to fluid being driven out of the liver cells and that part of the decrease in outflow under the choline drug may be due to fluid being absorbed by the liver cells.

Can the circulation through the turtle's liver be reversed? For the mammal Baer and Roessler (1926) claimed to have established a reversal of the portal vein-hepatic vein circulation. In the present series of experiments on the turtle's liver the perfusion apparatus was specially designed to try out the effects of reversing the direction of the perfusing fluid through the organ. Many attempts were made on several preparations under a variety of conditions but no fluid ever could be made to pass through the liver from the hepatic side out through the portal vein. To explain this in the absence of thrombi implies the presence of venous valves. But if there are no valves in the mammal's liver why should there be in a reptile's liver? The question seems to be important enough to admit of a detailed description of the apparatus used and the results obtained in the experiments.

The oncometer is provided with three three-way glass stopcocks welded in the pathways connecting the reservoir with the liver chamber. These are placed just outside the wall of the chamber. By manipulating the stopcocks the direction of flow from the reservoir and through the liver can be changed reversibly (portal vein $\rightleftharpoons$ hepatic vein) within a few seconds and as often as one pleases.

Records have been made of the reaction of the turtle's liver to these reversals of perfusion direction, illustrations of which appear in part I-a and I-b of figure 2 and in the graphs of figure 3.

Following first the drum record in figure 2 one notes (see arrow at top) that the preparation gives the expected slight response to small doses of AβMC, decrease in both rate of outflow and liver volume; the preparation is active. Otherwise the volume of the liver keeps at a steady level. When, at the arrow marked *Hep. V.* the direction of flow of the perfusate is changed to enter the hepatic vein and if possible to flow out of the portal (head of inflow pressure remaining always the same) the liver volume increases suddenly and rapidly and the outflow is stopped completely. The increase in liver volume continues until the limit of turgor apparently is reached, but ouflow is not established. Upon turning the cocks, so that the perfusate enters the portal vein (note arrow marked *P. V.*) and fluid may flow out of the hepatic vein again, then at once the outflow is recommenced and fluid freely flows out of the reservoir again.

This reversal is repeated, as the record shows in I-b of figure 2, and for a longer period. During this time adren.-HCl is injected but without avail. The greatest increase of liver volume (due doubtless to hepatic

sinus dilatation) occurring during the experiment (see fig. 3) is *ca* 19 ml. This would represent at least 50 per cent of the original volume of the active liver substance. But distention of the hepatic sinuses appears to

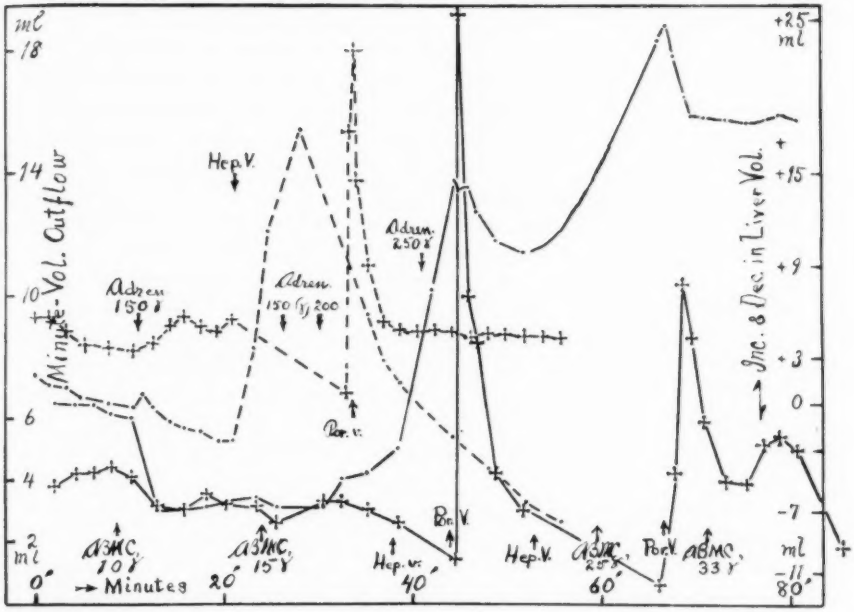

Fig. 3. This chart (expt. of Jan. 22) shows the behavior of the liver preparation in the oncometer as to changes in organ-volume (●, ●, ●), and minute volume outflow (+, +, +) under a steady head of inflow pressure. The pair of curves in full line show the response 1, to AβMC injected into the inflow-path, and 2, to reversal of the inflow fluid from portal vein to hepatic vein and then again suddenly returning the inflow back to the portal vein (note the arrows on the chart indicating these procedures and marked respectively *AβMC*, *Hep. V.*, and *Por. V.*). The reversal is carried out twice in the series of curves in full line.

On the same preparation and following immediately the above a similar series of experiments was carried out injecting adrenalin-HCl (Parke, Davis & Co.) instead of the choline drug; the results are plotted in the pair of curves filled out in broken line.

For both pairs of curves the abscissae mark the time in minutes, but *in reading the absolute volumes for the pair of curves in broken line, one should reverse the position of the legends as stated on the ordinates.*

be all that one can accomplish by reversing the circulation. Outflow from the liver through the portal vein is never established. AβMC, as well as adren.-HCl, fails to help. These experiments were repeated on several preparations and at various levels of inflow pressure, with and

without drugs, and always ending in failure to establish a reversed circulation. Whether the barrier was on the hepatic side, in the capillary bed or in the portal venules was not determined. In any explanation of this failure to reverse the circulation one has to consider the possibility of the presence of thrombi.

It is conceivable that clots could form at the mouths of hepatic venules so attached that they act as valves, open so long as fluid flows from the capillary bed toward the larger hepatic veins but closed completely when fluid begins to move from the larger hepatic veins back toward the capillary bed. The answer to this is 1, that the preparations were examined carefully at the end of the experiments for the presence of clots along the pathways of the veins and their branches but no macroscopic evidence was ever found of any clots. Microscopic sections were not made. 2. As described at the outset in this paper, and to forestall clotting, the perfusion fluid was sent through the liver at once upon inserting the cannula; in fact to exclude air emboli the flow from the cannula was begun very slowly before its tip was inserted in the vein. The hepatic outlet also was kept clear of clots and air bubbles. 3. After the cannula was in place the perfusate was continued for at least ½ hour, often longer, until the fluid flowed out of the organ quite clear before one began an experiment. The acacia in the perfusate keeps protein from leaving the tissues and entering the vascular bed. 4. The collected samples after such thorough washing never exhibited the presence of clots.

The experiments here reported failed to demonstrate that the venous circulation through the turtle's liver is reversible as has been reported for the mammal's liver. This suggests that histological search may reveal the presence of venous valves in the turtle's liver, but until such positive evidence is at hand a final conclusion must be withheld.

SUMMARY

1. An oncometer suitable for surviving small organs is described; also the preparation of a lobe of the turtle's liver and the manner of placing it in the oncometer without serious interruption of perfusion.

2. Experiments are described with this preparation in the oncometer, during which marked volume changes of the organ together with changes in rate of hepatic outflow are evoked with acetyl-β-methylcholine and adrenalin-HCl. These volume changes are graphically recorded.

3. A method of estimating inflow volumes for short-lasting time units, synchronous with observed outflow volumes, is described and its implications and validity discussed. By this method of estimating inflow volume and comparing the difference between inflow and outflow volumes it appears that in response to a choline drug the latter may be as much as 50 per cent greater or less than the former. This difference alone would

represent an enormous difference between input and output of substances for an organ (during identical time units) into the general circulation, and therefore may play an important rôle in the body economy.

4. In response to acetyl-β-methylcholine, injected into the portal inflow, the change in volume among ten preparations was usually a decrease, which at its maximum varied from 0 to 21.7 per cent of the liver preparation's volume at start. This decrease is usually followed by an increase during recovery that often is greater than the decrease evoked. (See table 2.)

5. In response to epinephrin the change in liver volume also tends to be negative in character but to a somewhat lesser extent perhaps. Among nine different preparations the maximum response in two cases was positive and of 16.5 and 9.3 per cent respectively; in the seven other cases the maximum response was negative, the decreases in volume varying from 1.3 to 22.8 per cent. (See table 2.)

6. Several attempts were made to reverse the perfusion direction so that fluid would flow into the hepatic vein and out of the portal vein, but never with any success. The precautions taken against clots and air emboli collecting in the vascular spaces were so thorough that it is considered most improbable that the failure can be attributed to emboli acting as one-way valves. No search has yet been made for venous valves however and the explanation for the failure is held in abeyance.

REFERENCES

BAER, R. AND R. ROESSLER. Arch. f. exper. Path. und Pharmakol. **119:** 204, 1926.

BAUER, DALE AND RICHARDS. 1929. Quoted after C. H. BEST and E. W. MCHENRY, Physiol. Rev. **11:** 423, 1921.

BLALOCK, A. AND M. F. MASON. This Journal **117:** 328, 1936.

BOWDITCH, H. P. Proc. Am. Acad. of Arts and Sci. **15:** 22, 1879.

BURTON-OPITZ, R. This Journal **36:** 330, 1915; Text-book of physiology, 1920, p. 397.

ERLANGER, J. AND H. GASSER. Ann. Surgery **69:** 397, 1919.

FORSGEN, E. Über die Rhythmik der Leberfunktion des Stoffwechsels u. des Schlaffes. Stockholm, 1935.

LAMPE, W. Arch. f. exper. Path. und Pharmakol. **117:** 92, 1926.

LAMPE, W. AND MEHES. Ibid. **119:** 66, 113, 1927.

LAWSON, P. D. AND J. ROCO. J. Pharmacol. Exper. Therap. **17:** 481, 1921.

LUNDSGAARD, E. Bull. Johns Hopkins Hosp. **63:** 90, 1938.

MAUTNER, H. UND E. P. PICK. Arch. f. exper. Path. und Pharmakol. **97:** 306, 1923.

SNYDER, C. D. AND R. E. JOHNSON. Bull. Johns Hopkins Hosp. **62:** 110, 1938.

SNYDER, C. D., R. E. JOHNSON AND C. MCI. PEEK. This Journal **124:** 704, 1938.

THE EFFECT OF DIURESIS ON THE EXCRETION OF GLUCOSE IN THE NORMAL DOG DURING CONSTANT GLUCOSE INFUSION

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The effect on urinary sugar of varying the quantity of water injected into and eliminated by normal animals during the intravenous infusion of constant quantities of glucose has been examined by Sansum and Woodyatt (1) and Davis (2). The former authors include a single protocol of the experimental results on one animal in which they found no change in glucose utilization or excretion; however, the degree of diuresis provoked was not particularly large. Davis, using 5 to 80 per cent solutions of glucose, observed that large quantities of water given with the glucose increased its retention by the body, so that less sugar was excreted despite diuresis. This result was attributed to a less precipitous rise in blood sugar with dilute glucose solutions and to the retention of water and accompanying glucose by the animal body. It was concluded, however, that if more water is available, more glucose is excreted into the urine at a given level of blood sugar than when less water is available.

In view of the limited number and differing results of available observations on the effect of diuresis on the glucose utilization of normal animals further investigation of this problem has been undertaken.

EXPERIMENTAL. Well nourished female dogs were used in these experiments. Each animal was fasted for 24 hours prior to glucose infusion. A cannula, attached by means of a rubber tube to a reservoir of saline solution, was inserted under local anesthesia into either a femoral or external jugular vein and was tied securely in place. In order to restrain movements by the animal which might result in dislodgment of the cannula, a molded plaster jacket was placed around the hind limbs and pelvis or forelimbs and neck prior to cannulation, which was accomplished through a window cut through the plaster. This completed plaster mold caused no particular discomfort to the animal which rested well cushioned on a catheterization board and indeed even fell asleep for short periods of time as the experiment progressed.

All operative procedures were performed under aseptic conditions and all solutions used and their containers were sterilized prior to the infusion.

A concentrated glucose solution of known strength was led from a burette through a pump, designed to deliver a small but constant volume of solution every second, and entered the animal's circulation through a hypodermic needle inserted into the lumen of the cannula leading from the reservoir of saline solution. Thus by keeping the flow of glucose concentrate constant, its dilution could be varied by adjusting the simultaneous flow of 1.0 per cent saline solution from the reservoir.

In view of the work of Jordan (3) and Wierzuchowski and Gadomska (4) showing that incoming glucose acts as a stimulus to the islet cells of the pancreas, thereby gradually increasing the tolerance to an optimum level, glucose was infused as a means of "priming" prior to any collection of urine for analysis, for thirty minutes at the subtolerance rate of 0.6 to 0.7 gram per kilogram of body weight per hour and was then elevated for thirty minutes to approximately 2 grams per kilogram per hour. Continuing the glucose infusion at the elevated rate, 1 or 2 one-hour control periods were then taken prior to creating a marked diuresis by the simultaneous infusion of saline solution. The subsequent saline infusions were made at rates of either 50 or 100 ml. per kilogram of body weight per hour.

Urine was collected for analysis at hourly intervals by means of a retention catheter and the bladder was washed out at the beginning and end of each period with 20 ml. of boric acid solution. Blood for sugar analysis was drawn either from an external jugular vein or a femoral artery, the site of withdrawal being the same for any one animal.

Urinary sugar and blood sugar were determined by the method of Shaffer and Somogyi (5), the filtrate for blood sugar analysis being obtained by the precipitation of blood with zinc hydroxide (6).

Results. Results of experiments on four animals are embodied in table 1.

Dogs I, II and IV showed definite increases in the total quantity of glucose excreted in the urine as the result of diuresis. Thus dog I, during the second hour of saline infusion, increased its sugar excretion from a control average of 0.61 gram to 7.58 grams. This in part may have been due to a rise in the blood sugar level that occurred during the first hour of saline infusion but evidently only in part for during the fourth hour of saline infusion (sixth hour in the table) when the blood sugar had again fallen to, or slightly below, the level of the control periods 3.93 grams of glucose were excreted. In dog II, whereas the blood sugar showed neither an upward nor a downward trend, the urinary sugar excretion during the second hour of saline infusion was 5.5 grams as compared to the control period average of 2.85 grams.

Dog III showed no very marked increase in sugar excretion, the output rising in the third hour of saline infusion to only 1.85 gram as compared

to a control period average of 1.27 gram. However, this rise, though slight, was accomplished during a definite blood sugar decline. Just why the blood sugar fell so markedly in this animal as the experiment progressed

TABLE 1

The effect of diuresis on the excretion of glucose in the normal dog during constant glucose infusion

DOG NUMBER	HOUR NUMBER	GLUCOSE INFUSED	TOTAL VOLUME INFUSED	GLUCOSE IN URINE	VOLUME OF URINE	BLOOD SUGAR*	GLUCOSE RETAINED PER KILOGRAM BODY WEIGHT	GLUCOSE EXCRETED PER KILOGRAM BODY WEIGHT
		grams	*ml.*	*grams*	*ml.*	*mgm. per cent*	*grams*	*grams*
I. Weight = 16.6 kgm.	1	33.1	97.4	0.52	13	196–182	1.96	0.03
	2	33.0	97.0	0.70	8	182–191	1.95	0.04
	3	33.1	937	3.88	360	191–236	1.76	0.23
	4	33.0	937	7.56	695	236–222	1.53	0.46
	5	32.9	1773	4.41	1215	222–184	1.72	0.27
	6	32.9	1773	3.93	1480	184–175	1.75	0.24
II. Weight = 8.4 kgm.	1	17.3	92.9	2.72	70	284–330	1.74	0.32
	2	17.3	92.6	2.98	122	330–313	1.71	0.35
	3	17.0	931	4.42	448	313–322	1.50	0.53
	4	17.2	932	5.50	680	322–291	1.39	0.65
III. Weight = 7.05 kgm.	1	13.2	88.0	1.31	40	346–330	1.69	0.19
	2	13.3	88.9	1.22	93	330–308	1.72	0.17
	3	13.4	450	1.61	168	308–246	1.68	0.23
	4	13.5	450	1.72	405	246–219	1.68	0.24
	5	13.5	790	1.85	628	219–	1.65	0.26
IV. Weight = 12.5 kgm.	1	24.8	93.9	0.77	145	209–202	1.92	0.06
	2	24.8	754	1.57	280	202–206	1.86	0.13
	3	25.1	755	3.18	615	206–198	1.75	0.25
	4	25.0	1315	3.80	1100	198–189	1.70	0.30
IV. Weight = 12.3 kgm.	1	20.2	80.7	0.064	23	200–189	1.64	0.005
	2	19.9	679	1.04	415	189–193	1.54	0.09
	3	19.7	1278	1.91	710	193–183	1.45	0.15
	4	19.8	78.9	1.89	420	183–184	1.45	0.15
	5	19.8	78.8	1.28	270	184–182	1.50	0.10
	6	19.7	78.5	1.36	235	182–180	1.49	0.11

* In dogs I, II and III blood sugar determinations were made on venous blood. In dog IV, arterial blood was used.

is not known inasmuch as it received the same experimental and pre-experimental treatment as was accorded the other animals.

Dog IV, in which the blood sugar varied but slightly throughout the

experiment, also showed a definitely increased sugar excretion as the result of diuresis. In a second experiment on dog IV a lower rate of glucose infusion was used, namely, 1.6 gram per kilogram per hour, and the urine excretion and blood sugar were followed for three hours after cessation of saline infusion, the glucose infusion being continuous. Although the sugar excretion decreased, as the diuresis became reduced, neither the sugar excretion nor the urine volume had returned to normal within this period.

In all of these experiments there was some lag between the infusion of saline solution and its excretion but this did not seem to encourage retention of glucose by the animal body as has been suggested by Davis (2).

Discussion. Three of the four animals used in these experiments gave results differing markedly from those reported by Sansum and Woodyatt (1) and Davis (2) in that they showed increased sugar excretion as the result of diuresis during a period of constant glucose infusion. It is probable that the degree of diuresis provoked in Sansum and Woodyatt's experiment was insufficient to bring about this effect. The difference between our results and those of Davis may in part have been due to difference in experimental procedure, namely, pre-experimental stimulation of the islet cells of the pancreas.

It does not seem necessary to postulate an altered carbohydrate metabolism to account for the increased excretion of sugar accompanying diuresis inasmuch as changes in the volume of glomerular filtrate may well have occurred. Walker et al. (7) found no increase in renal blood flow or glomerular filtrate during diuresis resulting from ingested water. However, the route of administration and degree of diuresis produced may not have been such as to bring into play the same mechanisms as were operative in our experiments.

Two changes which may well have occurred in these experiments to increase glomerular filtrate are 1, the opening of additional glomeruli, and 2, increased filtration through any given glomerulus. In previous experiments (8) it has been shown that saline infusions produce some increase in blood pressure. Shannon and Fisher (9) have recently shown that the essential limitation in the reabsorptive process lies in the circumstance that the tubules are able to transfer only a certain maximum quantity of glucose from the tubular urine to the blood per unit time. Inasmuch as the appearance of glucose in the urine during control periods in these experiments indicates that the blood sugar was sufficiently high so that more glucose entered the tubules than could be reabsorbed, the opening of additional glomeruli at the same blood sugar level would undoubtedly augment the excretion of sugar. Increased filtration through each glomerulus would have the same effect.

CONCLUSIONS

1. Intravenous saline infusions superimposed on constant glucose infusions (2 grams of glucose per kilogram of body weight per hour) by virtue of the resultant diuresis both decrease the quantity of glucose retained by the body and increase the quantity of glucose lost in the urine.

2. The increased excretion of glucose under the experimental conditions here described is believed to be the result of increased glomerular filtration.

REFERENCES

(1) Sansum, W. D. and R. T. Woodyatt. J. Biol. Chem. **30:** 155, 1917.
(2) Davis, D. M. This Journal **43:** 514, 1917.
(3) Jordan, E. M. This Journal **80:** 441, 1927.
(4) Wierzuchowski, M. and H. Gadomska. Biochem. Ztschr. **191:** 198, 1927.
(5) Shaffer, P. A. and M. Somogyi. J. Biol. Chem. **100:** 695, 1933.
(6) Somogyi, M. J. Biol. Chem. **86:** 655, 1930.
(7) Walker, A. M., C. F. Schmidt, K. A. Elsom and C. G. Johnston. This Journal **118:** 95, 1937.
(8) Cutting, R. A., A. M. Lands and P. S. Larson. Arch. Surg. **36:** 586, 1938.
(9) Shannon, J. A. and S. Fisher. This Journal **122:** 765, 1938.

THE RATES OF ABSORPTION OF THE RADIOACTIVE ISOTOPES OF SODIUM, POTASSIUM, CHLORINE, BROMINE, AND IODINE IN NORMAL HUMAN SUBJECTS[1]

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The discovery of artificial radioactivity and the preparation of radioactive isotopes of most of the known elements have given the biological sciences a new means of approach to study of the metabolism of living organisms. Chemically and physiologically, these artificially prepared radioactive isotopes do not differ from those that occur in nature, and they are inactive unless the concentration of the radioactive atoms is so great that changes are effected by the action of their radiations. The activated atoms can be considered as being tagged, and it is assumed their course in the body is an index of the metabolism of the inactive isotopes since the quantity of radioactive material used is extremely minute in comparison with the intensities required to produce biological changes.

The rates of absorption of radio-sodium[2] in a group of normal human subjects were reported recently (1). Following these experiments, the rates of absorption of radio-potassium, radio-chlorine, radio-bromine, and radio-iodine were determined in the same manner, but under more carefully controlled conditions. Further studies with radio-sodium also were made, conforming with the methods of administration of the other four radio-elements.

The purpose of this investigation has been not only to use this new method for the study of the metabolism of these elements in normal individuals; but also to establish an average normal value for the rate of absorption of each substance so that it might be compared with values obtained in similar experiments done on patients whose metabolism of one or more of these elements was altered. In this manner a new diagnostic means can be added to the resources of medical science.

PREPARATION OF THE RADIOACTIVE ISOTOPES. The radio-elements used in this series of experiments were prepared by use of the magnetic resonance

[1] This work has been supported by the Josiah Macy Jr. Foundation and the Christine Breon Fund.

[2] The prefix, "radio-" indicates that the element so described is radioactive.

accelerator developed by Lawrence and Livingston (2) and later improved by Lawrence and Cooksey (3). This apparatus, commonly termed the "Cyclotron," has been developed for the multiple acceleration of light ions to high speeds. The ions spiral around within a drum-shaped chamber lying between the poles of a powerful electromagnet. The combined action of the magnetic field and a high frequency oscillating electric field upon these particles produces progressive increments of velocity with every revolution of the ions within the chamber so that their terminal energy upon emerging from the periphery ranges up to 16,000,000 electron volts. These particles stream out of the cyclotron as a narrow beam which is directed against the substance to be activated.

Deuterons, which are the ions of heavy hydrogen, were used to prepare the radio-sodium, radio-potassium, radio-chlorine, and radio-bromine. The deuterons, each of which consists of a proton and neutron bound together, are accelerated in the cyclotron to an energy of 8,000,000 electron volts. When one of these swiftly moving particles approaches an atom of the substance to be activated, the deuteron becomes distorted in such a manner that the neutron is separated from the proton; the neutron enters the atomic nucleus whereas the proton is repelled from the intensely positive nuclear field. This process is termed "neutron capture" and results in the formation of an isotope which is heavier by the added weight of the neutron. Such a reaction can be produced in most of the known elements, and in many instances results in the formation of isotopes which do not occur in nature and which are energetically unstable. These artificially-produced, unstable atoms are radioactive. When they disintegrate, the nucleus of each atom expels an electron, i.e., beta ray. The loss of the electron from the atomic nucleus changes the nuclear charge and therefore results in a transmutation of the element. When a negative electron is discharged, the atomic nucleus gains a positive charge of one without altering its mass, and its atomic number increases by one. Frequently the nucleus retains an excess of energy after the expulsion of the electron, and in such instances a penetrating gamma ray is emitted immediately. All five of the radio-elements used in these experiments yielded strong gamma rays. Figure 1 indicates the nuclear reactions for the formation and disintegration of each of these five radio-elements. The numerical subscripts indicate the atomic numbers, and the superscripts the atomic weights. The final disintegration products of these activated atoms are of no biological significance since the quantities formed are too minute to be detected. The half-life,—that is, the length of time required for half of a given number of activated atoms of a radio-element to disintegrate,—is specific for each substance and varies from only 0.88 second for radio-lithium to more than 10 years for radio-cobalt.

In addition to the deuteron method of producing activated elements,

there are several other projectiles which may be employed. Neutrons can be produced in large quantities by the deuteron bombardment of some of the lighter elements. In such cases the proton is captured by the atomic nucleus, and the neutron is set free. The element to be activated is placed in the path of the neutrons. In most instances, however, much larger yields of the radio-elements are obtainable by direct deuteron bombardment. Protons, the nuclei of ordinary or "light" hydrogen, and alpha particles which are the nuclei of helium atoms, also may be

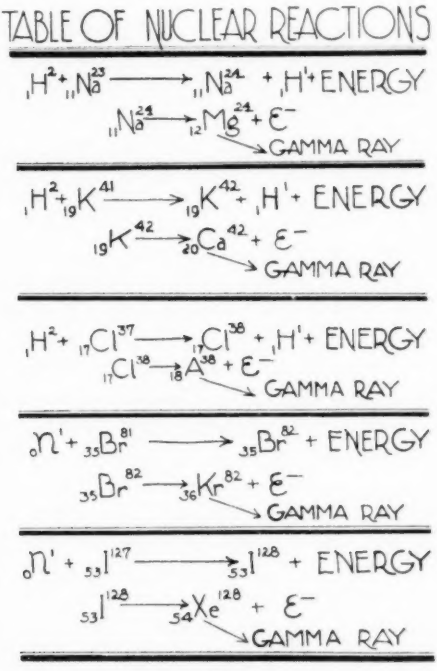
TABLE OF NUCLEAR REACTIONS
$_1H^2 + _{11}Na^{23} \longrightarrow _{11}Na^{24} + _1H^1 + ENERGY$
$_{11}Na^{24} \longrightarrow _{12}Mg^{24} + \mathcal{E}^-$
GAMMA RAY
$_1H^2 + _{19}K^{41} \longrightarrow _{19}K^{42} + _1H^1 + ENERGY$
$_{19}K^{42} \longrightarrow _{20}Ca^{42} + \mathcal{E}^-$
GAMMA RAY
$_1H^2 + _{17}Cl^{37} \longrightarrow _{17}Cl^{38} + _1H^1 + ENERGY$
$_{17}Cl^{38} \longrightarrow _{18}A^{38} + \mathcal{E}^-$
GAMMA RAY
$_0n^1 + _{35}Br^{81} \longrightarrow _{35}Br^{82} + ENERGY$
$_{35}Br^{82} \longrightarrow _{36}Kr^{82} + \mathcal{E}^-$
GAMMA RAY
$_0n^1 + _{53}I^{127} \longrightarrow _{53}I^{128} + ENERGY$
$_{53}I^{128} \longrightarrow _{54}Xe^{128} + \mathcal{E}^-$
GAMMA RAY

Fig. 1

accelerated in the cyclotron. These particles also are capable of inducing radioactivity in many elements; however, further discussion concerning them will be omitted since they were not employed in the preparation of the radio-elements used in these experiments.

The *radio-sodium* was prepared by the deuteron bombardment of 1.0 gram of NaCl for a few minutes. Both radio-sodium and radio-chlorine were formed, but since the half-life of the radio-chlorine is only 37 minutes the salt was allowed to age for several hours. At the end of this time the remaining radioactivity was due entirely to the radio-sodium which

has a half-life of 14.8 hours. The salt was then weighed and dissolved in enough water to make an isotonic solution.

The *radio-potassium* was prepared in a similar manner from 1 gram of KCl and made up as an isotonic solution. In this instance, the period of aging was a little longer since a prolonged bombardment was necessary to prepare the radio-potassium, and therefore more radio-chlorine was formed. However, only a small proportion of the radio-potassium was lost through decay during the ageing period since the half-life of this substance is 12.4 hours.

The *radio-chlorine* was made by the bombardment of 250 mgm. of LiCl. In this instance, the period of ageing was negligible since radio-lithium has a half-life of only 0.88 second, and very little of the radio-chlorine was lost as its half-life is 37 minutes. The salt was then dissolved in 40 cc. of water to make an isotonic solution.

The *radio-bromine* for the first five experiments in which this active isotope was used, was produced by the deuteron bombardment of 1.5 gram of KBr. The activated salt had to be aged for from 24 to 36 hours so as to allow the radio-potassium to decay away. This period of time was adequate since a much higher proportion of bromine than of potassium was activated. From $\frac{1}{3}$ to $\frac{1}{2}$ of the radio-bromine was lost through decay since its half-life is 34 hours. The activated salt was then converted to an isotonic solution. For the last three of the radio-bromine experiments, the activated element was made by exposing 1 kgm. of ethylbromide to an intense neutron source and by separating out the radio-bromine which had been formed. This method was first developed by Szilard and Chalmers (4) who, realizing that when an atom captured a neutron it recoiled with sufficient energy to break chemical bonds, applied this principle to the preparation of highly concentrated radioactive material. Much stronger samples of a few of the heavier elements, such as bromine and iodine, may be prepared by this method than by deuteron bombardment. Following the exposure of the ethyl bromide to the neutron source for several hours, the radio-bromine was extracted by adding 20 cc. of an aqueous solution containing 100 mgm. of KBr, 50 mgm. of Na_2SO_3 and 100 mgm. $NaHCO_3$ to the ethyl bromide. The mixture was shaken vigorously for several minutes, and the aqueous layer removed and boiled to drive off the traces of ethyl bromide present. Sufficient inactive KBr was added so as to make up 100 cc. of isotonic solution.

The *radio-iodine* was prepared in the same manner from 3 kgm. of ethyl iodide to which 200 mgm. of I_2 was added to act as a carrier. After exposure to the neutron source, 20 cc. of an aqueous solution containing 100 mgm. of Na_2SO_3 and 200 mgm. of $NaHCO_3$ were added, and the mixture was shaken for several minutes. The aqueous fraction was then separated and boiled so that the total volume was brought to 25 cc., which

thereby made the solution isotonic; but no more iodide was introduced. Just before administration, the gamma ray intensity of each sample was measured with a Lauritsen fiber electroscope which had been calibrated previously against a known radium standard. The gamma ray activities of the samples were expressed as μ gm.e.[3]

METHOD OF STUDY AND ADMINISTRATION. Eight normal human subjects were used in each of the five series of experiments. Whenever possible, the same individual was given more than one of the radio-elements being investigated. All of the subjects were in fasting state when the radioactive solutions were given. The amounts of radioactive material given to each subject varied from 50 μ gm.e. to 800 μ gm.e. This wide variation was unavoidable since more than 100 μ gm.e. of radio-potassium were difficult to prepare; and larger quantities of the short-lived radio-chlorine and radio-iodine were needed in order that the absorption curves might be followed as long as possible. All of the solutions administered were isotonic. In the case of radio-sodium, radio-potassium, and radio-bromine, 100 cc. of isotonic solution were used. This particular quantity was established because it was not feasible to activate efficiently less than 1.0 gram of KCl at a time. It was felt inadvisable to administer more than 250 mgm. of LiCl or NaI at a time because of the known tendency of these compounds to provoke gastric irritation, particularly when the stomach is empty.

The radioactivity of the hand was used as an indicator of absorption. The subject held in his hand a small glass-walled Geiger-Müller counter-tube. This was encased in a copper tube with walls of 1 mm. thickness, so as to screen out all of the beta rays. A large lead box with walls 5 inches in thickness and open at one end had been constructed, in which the counter-tube was held by the subject. This arrangement permitted the accurate measurement of very minute quantities of any radioactive substances in the hand, and at the same time shielded the counter-tube from the gamma radiation from the remainder of the subject's body.

The Geiger-Müller counter, which was used to measure the radioactivities of the subjects' hands, is an instrument which has been developed for the purpose of quantitative measurement of very small amounts of radioactive material. The counter consists essentially of a cylindrical copper cathode and an anode made of tungsten wire which is placed on the axis of the cylinder. The electrodes are sealed in a glass tube which is filled with a gas at reduced pressure. A potential of from 400 to 2000 volts is impressed across the electrodes, and when a gamma ray or beta particle enters the tube secondary electrons are generated in the walls of the counter or in the gas. These secondary electrons are rapidly swept out by the charged

[3] 1μ gm. e. equals the Gamma ray intensity of 1μ gm. of radium element in complete equilibrium with its decay products.

electrodes, and thus produce additional ions which create a small pulse of current. These feeble electric discharges can be counted by coupling the counter circuit to a vacuum tube amplifier which operates a mechanical recorder. With the instruments used in these experiments, it was possible to measure gamma ray intensities equivalent to less than 5×10^{-9} grams of radium element.

At the start of each experiment, the subject took his station with the counter-tube held firmly in one hand within the lead chamber, and the sample to be swallowed held in the other hand. A reading was then taken on the counter so as to determine the amount of radiation produced by cosmic rays; by the natural radioactivity of the lead, counter-tube, subject's hand, etc.; and by the activated sample. This value, which is defined as the "background," varied from 0.08 to 0.80 impulse per second depending upon the activity of the sample and the size of the counter-tube used. With the subject in a fasting state, the solution was then ingested, and, immediately after, from 10 to 15 cc. of water were swallowed for the purpose of washing all traces of the radioactive solution from the mouth and esophagus. Readings were then taken every few minutes for the next $2\frac{1}{2}$ to 3 hours, and the subject was then allowed to leave for lunch. At the end of $2\frac{1}{2}$ hours the activities of the radio-chlorine and radio-iodine had decayed to values but little greater than that of the background, at which point these experiments were terminated. When radio-sodium, radio-potassium, and radio-bromine were being used, the subjects returned and readings were continued until the end of from $6\frac{1}{2}$ to $8\frac{1}{2}$ hours. After every 10 minutes the subject withdrew his hand from the lead chamber, leaving the counter-tube within, and a background reading was taken. This was done so as not to allow the subject's hand to become cramped, thereby interfering with the circulation; and also in order to check the background readings.

On several occasions, two subjects were used together and under these circumstances they both drank the activated solutions at the same time. Readings were taken every 90 seconds for each subject for the first hour and every 5 minutes thereafter. The background values were determined in the usual manner. The room in which these experiments were done was kept at a uniform temperature.

Several corrections had to be applied to the measurements made with the counter. First the background value was deducted; next, allowance was made for the non-linearity of the counter; and, last, correction was made for the decay of the radio-element during the period of time that the experiment was being conducted.

When the counting-rate exceeded 20 impulses per second, the inertia of the mechanical recorder would not allow all of the impulses to be counted. In order to determine accurately the degree of non-linearity of various

counting speeds above the linear range, it was necessary to prepare several radioactive samples each of which gave at a given distance a counting rate of 20 impulses per second. The samples were then added (one at a time) to each other until the measured counting rate was greater than that which was to be encountered in any of the experiments. From these results a curve was drawn with the ordinates representing the measured rates of counting and the abscissa indicating the theoretical rates. This curve was then used to determine the corrections necessary for counting-rates above the linear range of the instrument.

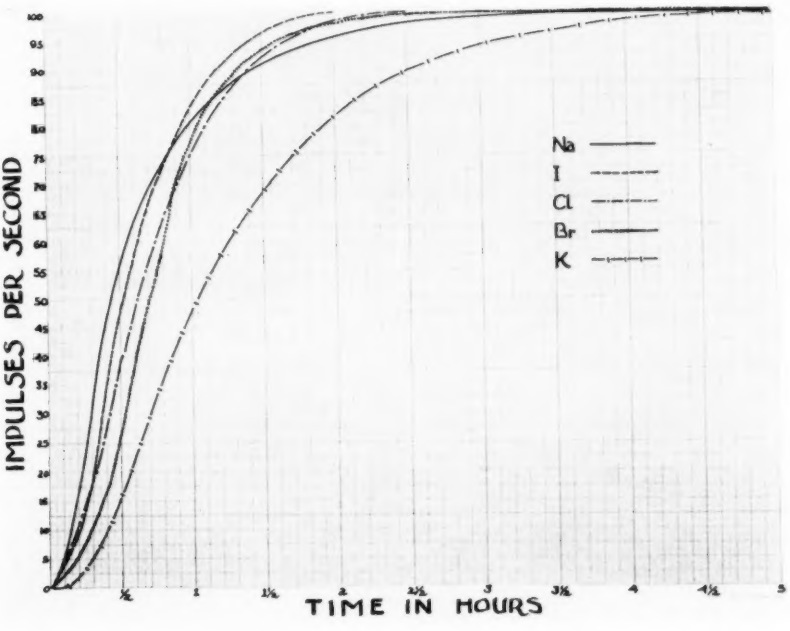

Fig. 2

In each experiment the individual readings were divided by the interval of time that they were taken so that they could be expressed as impulses per second. The average value for the background obtained in the same experiment was deducted; and, when necessary, correction made for the non-linearity of the counter. Each point was then divided by the decay factor for the particular radio-element used, the initial time being taken as the moment when the subject ingested the activated solution. The absorption curves were then plotted, the ordinates representing the activity of the hand in impulses per second, and the abscissa indicating the interval

of time after the subject received the radioactive solution. The curves thus obtained were so adjusted that the maximum height of each was set to a common value of 100. From these values composite curves were constructed for each of the five radio-elements studied (fig. 2).

Experimental results. Within 3 to 6 minutes following ingestion, the radio-sodium, radio-chlorine, radio-bromine or radio-iodine could be detected in the hand, and the activity increased rapidly thereafter. The radio-potassium was taken up more slowly, an interval of from 6 to 15 minutes being required before it appeared in the hand, and the rate of

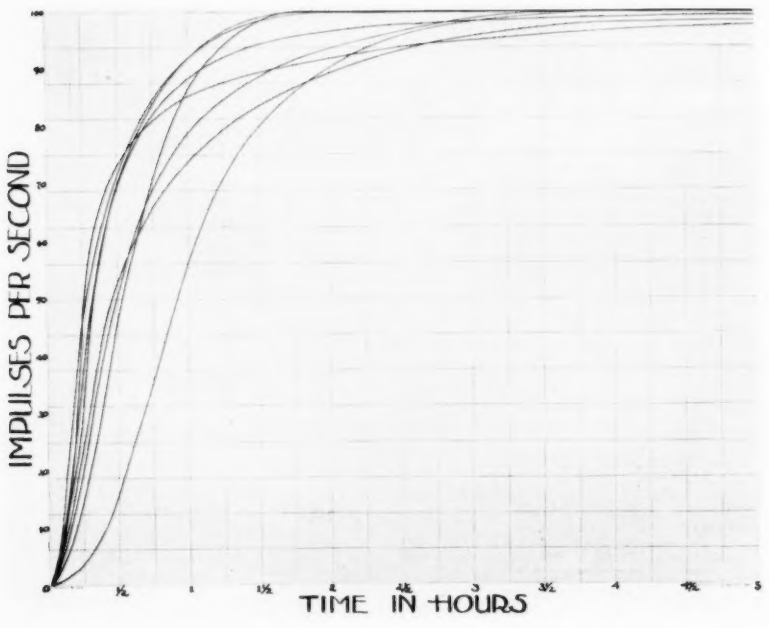

Fig. 3

increase of activity was slower than for any of the other four radio-elements. At the end of one hour after ingestion of the active solutions, the curve for radio-potassium had reached only 48 per cent of its final value, whereas the curves representing the absorption of the other four radio-elements had risen to from 75 to 85 per cent of the maximum height. In the case of radio-chlorine and radio-iodine, the exact establishment of this maximum point of the curves for each substance was subject to an error of several per cent, since the half-lives of these two elements were so short that during the final half-hour of the experiments the counting rates were very low. Under such circumstances the statistical fluctuations

of the number of counts taken for each point were of the order of from 5 to 10 per cent, and therefore the slopes of the terminal portions of the curves for these two substances are not as accurate as the same section in the curves of the other three elements. In all of the experiments it was noted that there was considerable individual variation in the values obtained for different subjects who were given the same radio-element. Figure 3 illustrates the variations observed in the eight subjects who received radio-sodium. The individual differences observed in the other four groups of experiments were of the same order of magnitude. These variations were not specific for the subjects upon whom two or more radio-elements were used. For example, a subject might show a rate of absorption for radio-bromine which was more rapid than the average and yet absorb radio-sodium more slowly than the mean rate for that substance. It must be remembered, however, that considerable individual variations occur in almost all quantitative metabolic measurements. When the absorption curves had reached a maximum for the radio-sodium, radio-potassium, and radio-bromine, they continued at this level for the duration of the experiments.

Discussion. Before these curves can be considered as quantitative indices of uptake of sodium, potassium, chlorine, bromine, and iodine, several factors must be analyzed. First, can the rate of change of radioactivity of the hand be taken as a measure of the rate of absorption of the radioactive atoms into the blood stream? To answer this question, one must visualize the probable course of events as the active material is transferred from the digestive tract to the blood, and then transported throughout the body. As soon as these atoms enter the circulation, a certain proportion of them will diffuse out of the blood into the surrounding tissues, including those of the hand that is holding the counter-tube. Therefore the radioactivity of the hand will be the sum of the disintegrating atoms in the tissues of the hand plus those in the blood flowing through it. At the same time, the total number of active atoms in the blood is being decreased by diffusion of some of the atoms into the tissues of the body. Now the proportion lost from the blood will decrease as more enter the tissues, until finally equilibrium is attained and the number moving in each direction will be constant. During the first hour after ingestion, the number of active atoms in the blood will increase rapidly so that the net effect will be a movement to the tissues. But when no more tagged atoms are being added to the blood from the digestive tract, an equilibrium will be eventually established; and when this state is reached the curve will become level. This assumption is, of course, not strictly valid for elements which are fixed in relatively high quantities in certain of the body's tissues, such as calcium and phosphorus in the skeleton.

Now if the subjects were hypothetical organisms whose tissues were of

homogeneous chemical and physiological properties, the curves would not be affected by the rate or degree of diffusion of the radio-elements from the blood into the tissues. This may be assumed because the blood vessels are so extensively distributed in the hand that the activity measured will be the same at a given moment regardless whether the active atoms are in the blood or the adjacent tissues. However, the human body is not a homogeneous organism and a given portion of it, such as the hand, may take more or less than its proportional share of the active atoms that the individual has swallowed. If the hand withdraws from the blood proportionately less of the active material than is taken up by the rest of the body, then the height of the curve would be less than if the hand acquired more than its share of the active atoms. Should the rate of diffusion be much more rapid than the rate of absorption, the only difference between these two situations would be in the height of the curves and they would reach their maximum values after the same interval of time and would both remain level thereafter. Should the rate of diffusion be slower and the tissues of the hand receive a smaller proportion of the active atoms, the curve would rise to its maximum and then decline as the rest of the body was withdrawing the active material from the blood, and not become level until equilibrium was attained. Should the tissues of the hand receive a larger proportion of the active atoms, then the curve would continue to rise after absorption was completed and would not become level until the diffusion had reached equilibrium.

When this theoretical analysis is applied to the experimental results in the cases of radio-sodium and of radio-bromine, one or more of three interpretations is possible. First, the hand receives the same proportional share of the active atoms as the rest of the body; second, the rates of diffusion are much more rapid than the rates of absorption; third, the rates of absorption are extremely rapid with the rates of diffusion of about the same value or slower, and the hand receives more than its share of the radio-sodium or radio-bromine. The second possibility seems to be the most probable, since in the first instance it is highly unlikely that the hand will withdraw from the blood the same relative quantities of these two radio-elements and in the last case it is not reasonable to assume that absorption is as rapid a process as diffusion. A similar interpretation for the results obtained with the radio-chlorine and radio-iodine is very probable; but since these experiments could be continued for only a short time after the curves reached their maximum values, a positive conclusion cannot be made. In the case of the radio-potassium, it is apparent that the rate of absorption is less than for the other radio-elements since the initial appearance of the active potassium atoms in the hand was delayed. Due to this observed fact, the character of the potassium curve can also be adequately explained by the interpretation given for the other substances.

The second question which presents itself in these studies is: can it be assumed that the metabolism of the different radio-elements is identical with the behavior of their non-radioactive isotopes? As has been mentioned previously, the chemical and physiological properties of the active and inactive isotopes of a given element are identical and therefore the course pursued by these tagged atoms may be assumed to be the same as that taken by their inactive isotopes.

From the preceding discussion it seems reasonable to conclude that the curves obtained for the rates of absorption of radio-sodium, radio-potassium, and radio-bromine are very similar to those of the rates of uptake from the digestive tract of sodium, potassium, and bromine; and that a similar interpretation is probable for chlorine and iodine.

SUMMARY

1. The preparation, administration, and method of measurement of the radioactive isotopes of sodium, potassium, chlorine, bromine, and iodine for the purpose of determining the rates of absorption of these five elements in normal human subjects have been described; and a discussion of the interpretation of the experimental results has been given.

2. The rates of absorption of sodium, chlorine, bromine, and iodine have been found to be quite rapid, the radioactive atoms of each of these four substances being detected in the hand within 3 to 6 minutes after ingestion. Absorption is apparently completed within 3 hours. Potassium was absorbed more slowly; the activated atoms were not detected until from 6 to 15 minutes following ingestion of the salt, and absorption was not completed until after 5 hours had elapsed.

3. The method described for the quantitative measurement of the rates of absorption of these five radio-elements in normal human subjects is applicable to other elements of biological importance and to compounds which can be synthesized from radioactive substances. Furthermore, the normal values obtained in these experiments and those which will be obtained in the future for other substances can be compared with those found in disease. Such comparisons will be important not only for a clearer understanding of the metabolic changes in disease but also as a diagnostic means.

Acknowledgments. The author wishes to express his appreciation to Prof. Ernest O. Lawrence and his co-workers in the Radiation Laboratory of the University of California for the preparation of the radio-elements used in these experiments and for their advice concerning physical measurements; and to the individuals who, as the experimental subjects, made these studies possible. The Geiger-Müller counters used in this work were designed and built by Dr. Ernest M. Lyman of the Radiation Laboratory. The suggestions and criticisms offered by Drs. John H. Lawrence and Mayo

H. Soley of the Department of Medicine, and Prof. Edwin McMillan of the Radiation Laboratory, are gratefully acknowledged. The experiments were materially facilitated by W. P. A. laboratory assistance.

REFERENCES

(1) HAMILTON, J. G. Proc. National Academy of Sciences **23**: 521, 1937.
(2) LAWRENCE, E. O. AND M. S. LIVINGSTON. Phys. Rev. **40**: 19, 1932.
(3) LAWRENCE, E. O. AND D. COOKSEY. Phys. Rev. **50**: 1131, 1936.
(4) SZILARD AND CHALMERS. Nature **134**: 462, 1934.

PILOCARPINE CONDITIONING

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Pilocarpine, like morphine, injected hypodermically in dogs evokes profuse salivation. Kleitman (2) and Crisler (1) have reported that repeated injections of pilocarpine, unlike morphine, do not result in the establishing of conditional salivary responses. This difference, apparently, is attributable to the fact that the two drugs have different loci of action. The sialagogue action of pilocarpine is *peripheral;* it presumably acts on the myoneural junction (Sollmann (6), p. 402). Morphine, as Kleitman and Crisler (3) have shown, produces a salivary secretion only as an accompaniment of a *centrally* aroused nausea complex. This seems to indicate that the exercise of a response does not in itself provide an adequate basis for conditioning that response. Recently Loucks (4, 5) has adduced evidence which complements this conclusion.

Since the negative conclusions of these experiments are of fundamental importance in understanding the mechanisms of CR formation, the present experiment was planned to provide a technically refined check on Crisler's and Kleitman's work. The fact that conditional salivary secretions are frequently so small that they might escape detection by the Kleitman-Crisler technique seemed to justify repetition of the work since facilities were available for the accurate measuring of very minute quantities of saliva (*cf.* 7). (The Kleitman-Crisler technique involves the direct collection and measurement in a centrifuge tube of the salivary secretion from the externalized Wharton's duct. The present technique utilizes an hydraulic tube system sealed to an externalized parotid duct, and the quantity of secretion is determined by measuring the displacement of an air bubble in a calibrated capillary tube. By this technique, measurements of 0.01 ml. saliva are reliable.)

Four dogs without previous CR experience were prepared with parotid fistulae. After the animals had fully recovered from the fistulae opera-

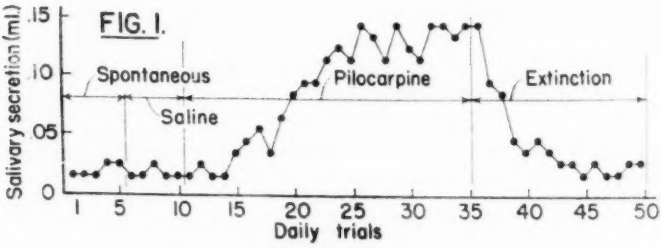
[1] Yale Laboratories of Primate Biology. This investigation was conducted while the writer was a National Research Council Fellow, Division of the Biological Sciences. Grateful acknowledgment is made to Dr. W. H. Gantt for his counsel throughout the experiment.

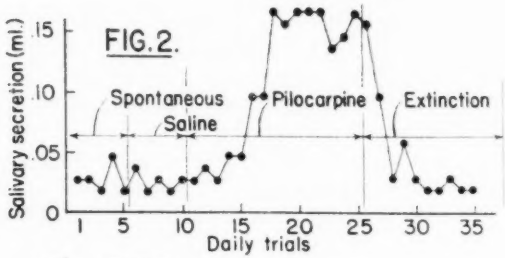
tions, they were placed in the usual CR apparatus and daily preliminary observations were begun. These preliminary observations, which serve as comparison and control for the later critical observations, consisted of 5 daily 30 minute parotid secretion measurements with subjects given 1 ml. subcutaneous injections of normal saline solution 15 minutes after being placed in the apparatus.

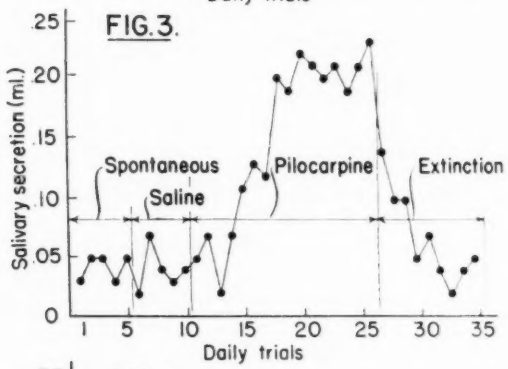
After completion of these preliminary observations, injections of 1 ml. aqueous solution of 10 mgm. pilocarpine hydrochloride were substituted for the saline solution injections. This technique was continued with animal number 1 for 25 days, animal number 2 for 15 days, animal number 3 for 16 days, and animal number 4 for 25 days. After these periods had elapsed, the pilocarpine injections were discontinued and the salivary secretion measurements without external stimulation resumed.

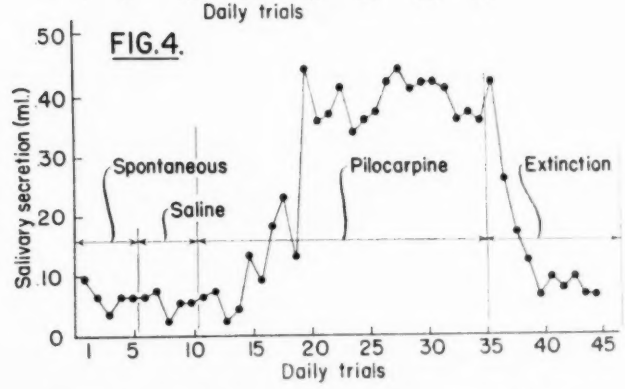
The results for animals 1 to 4 are presented in figures 1 to 4 respectively. The points on the curves represent the volume of saliva secreted during the *first* 15 minutes the animal was in the apparatus, *i.e.*, *before* injection. In every case, the secretion during the "spontaneous" and saline injection periods is low (less than 0.1 ml.); during pilocarpinization the quantity increases progressively for about 10 days when it reaches a maximum. This maximum persists until the pilocarpine injections are discontinued and then the volume drops for about 10 days until the normal "spontaneous" level is again reached.

Several alternative hypotheses might account for the increased salivation during the pilocarpinization periods: *1.* The effect may be due to a progressive slowing of the process of pilocarpine-elimination from the body, so that what appears as pre-injection secretion is actually post-injection secretion (from the previous day's injection). *2.* Perhaps chronic pilocarpinization results in a peripheral hypersensitizing, so that efferent impulses which are normally ineffectual in eliciting secretion become more and more effectual. *3.* Perhaps the effect is due to conditioning, but, like morphine-conditioning, is really a secondary effect occurring only as an accompaniment of conditional nausea. *4.* Perhaps this is an example of conditioning which is dependent only on exercise of a specific response. Of these alternatives, the second and fourth seem most plausible. The first hypothesis can hardly account for the effect's persisting for a few days after withdrawal of pilocarpine. Examination of the rates of secretion during the periods of maximal response shows that the secretory activity, from the moment of introducing the animal into the apparatus until the time of injection, is positively accelerating. If the effect were due to the slow elimination of the previous day's pilocarpine it would seem that the rate should be either negatively accelerating or constant. The third hypothesis, *viz.*, that the effect is a secondary accompaniment of conditional nausea, seems unsatisfactory because objective manifestations of nausea were seldom observable and because the small volumes of secre-









Figs. 1–4. Daily volumes of salivary secretion, animals 1 to 4

tion elicited are incomparable to the great volumes reported in morphine-nausea-conditioning experiments.

Since the present reported increased salivation is so small and since this experiment does not provide further data for identifying the mechanism which is operative, it seems that the only conclusion justified is that the question as to whether or not exercise alone provides an adequate basis for conditioning must remain open.

CONCLUSIONS

1. Daily subcutaneous injections of 10 mgm. pilocarpine hydrochloride solution results in a small progressive heightening of the preinjection parotid salivary secretion (4 dogs).

2. Withdrawal of pilocarpine results in the returning of the secretory volume to the normal "spontaneous" level.

3. These results, while not *proving* that exercise of a response provides alone an adequate basis for conditioning that response, indicate that the question as to the efficacy of exercise in conditioning must be regarded as still open.

REFERENCES

(1) Crisler, G. This Journal **94:** 553, 1930.
(2) Kleitman, N. This Journal **82:** 686, 1927.
(3) Kleitman, N. and G. Crisler. This Journal **79:** 571, 1927.
(4) Loucks, R. B. J. Psychol. **1:** 5, 1936.
(5) Loucks, R. B. J. Psychol. **4:** 295, 1937.
(6) Sollmann, T. A manual of pharmacology. Philadelphia: W. B. Saunders Co., 1934.
(7) Wolff, H. G. and W. H. Gantt. Arch. Neurol. and Psychiat. **33:** 1030, 1935.

THE EFFECT OF VITAMIN B_1 AND THE VITAMIN B_2 COMPLEX ON THE WEIGHT, FOOD INTAKE, AND ESTRUAL CYCLE OF HYPERTHYROID RATS

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Himwich, Goldfarb and Cowgill (10) reported that dogs fed thyroid gland required an increased amount of undifferentiated vitamin B. Cowgill and Palmieri (3) obtained similar results with pigeons fed thyroid gland. Later Sure and Buchanan (14) were able to *prevent* hyperthyroidism with vitamin B_1, as judged by the gain in weight, after the stable components of the vitamin B_2 complex had been provided. Drill (5) reported that hyperthyroid rats which had lost weight would *regain* their lost weight, while still receiving thyroid gland, only when both vitamin B_1 and a yeast concentrate were given. Crystalline vitamin B_1 alone did not enable the hyperthyroid rats to regain their lost weight. A sex difference in response was also reported. Further studies on the effect of crystalline vitamin B_1 and the vitamin B_2 complex on experimental hyperthyroidism are being reported.

This study was designed as follows: 1, to feed rats a synthetic diet containing sufficient amounts of yeast for growth; 2, to feed thyroid gland to rats receiving the same diet and produce a loss of weight; 3, crystalline vitamin B_1 and additional yeast were then administered to the hyperthyroid rats, while they were still receiving thyroid gland, in an effort to enable them to regain their lost weight. This will determine any antithyrogenic action, as judged by the gain in weight, that crystalline vitamin B_1 and yeast may have on experimental hyperthyroidism in rats.

METHODS. The rats used in these experiments were raised on Purina Dog Chow and for the experiments were transferred to one of the diets listed in table 1. The vitamin B_1 used was Merck's synthetic vitamin B_1. Yeast no. 15190 is a dried brewer's yeast containing 18 International Units of vitamin B_1 per gram and 20 Sherman-Borquin Units of vitamin G (flavin) per gram. Yeast no. 15190 is relatively low in the other factors of the vitamin B_2 complex. Yeast no. 15549 is a brewer's yeast concentrate (rich in the vitamin B_2 complex) containing 55 I.U. vitamin B_1 per gram. Yeast no. 17000 is a dried yeast, also rich in the vitamin B_2 complex, but

low in vitamin B_1, containing only 6 I.U. vitamin B_1 per gram. The thyroid gland is U. S. P. material containing 0.2 per cent iodine.[1]

Experiment 1. In this experiment female rats, weighing about 250 grams, were fed diet 8 ad libitum, which is a synthetic diet plus a daily supplement of 200 mgm. of yeast no. 15190 per day. This supplied each rat with 3.6 I.U. vitamin B_1 and 4 S-B. units of vitamin G per day. The changes in weight are shown in figure 1. The positive controls (group 1), 5 rats, which received only diet 8, gained weight. The negative controls (group 2), 5 rats, received diet 8 plus 100 mgm. thyroid gland per day, and showed a rapid loss of weight. The 12 test rats (group 3) also received diet 8 plus 100 mgm. of thyroid gland per day. These rats also showed a rapid loss of weight. On the 17th day of thyroid feeding a daily subcutaneous injection of 500 gamma of vitamin B_1 was begun in group 3. The rats in group 3, as will be seen in figure 1, did not lose any more weight

TABLE 1

Composition of the diets

DIET NUMBER	COD LIVER OIL (USP)	SALTS*	CRISCO	CASEIN	CORN-STARCH	YEAST†
5	2	4	10	20	59.0	5.0
6	2	4	10	20	54.0	10.0
8	4	4	10	20	62.0	0.0‡
9	4	4	10	20	59.5	2.5

* Osborne and Mendel salt mixture.

† Yeast no. 15190.

‡ Plus a supplement of 200 mgm. of yeast no. 15190 per day.

when injected with vitamin B_1. The rats in group 3 remained constant in weight and did not regain any of their lost weight. The 200 mgm. of yeast no. 15190 fed to each rat per day do not supply very much of the vitamin B_2 complex. Accordingly, on the 26th day the test rats were divided into 2 groups (groups 3 and 3A); six rats in each group. Group 3 was continued on the same diet as the group 3 above. In group 3A the only change was the substitution of 5 per cent of yeast concentrate no. 15549 for 5 per cent of the starch in diet 8. Each rat in group 3 and 3A still received 100 mgm. of thyroid gland and 500 gamma of vitamin B_1 per day.

The rats in group 3A (fig. 1) began to gain weight as soon as the yeast concentrate was added to the diet, whereas the rats in group 3 showed

[1] No iodine, other than that in the salt mixture and in the thyroid gland, was added to the synthetic diets. The authors wish to thank Mr. Light of the Fleischmann Laboratories for supplying the vitamin B_1 and the analyzed yeast, and Doctor Rhodehamel of Eli Lilly and Co. for supplying the thyroid gland.

neither a gain nor a loss of weight. It is evident, therefore, that certain other factors or vitamins of the B_2 complex present in the yeast concentrate are necessary to enable rats injected with vitamin B_1 to regain the amount of weight lost, while still receiving thyroid gland. The amount of the vitamin B_2 complex in yeast no. 15190 is low, but judging by the gain in weight of the positive controls, is sufficient for normal growth. During experimental hyperthyroidism, however, this amount of the B_2 complex was too small to enable the rats to regain any lost weight when injected with vitamin B_1.

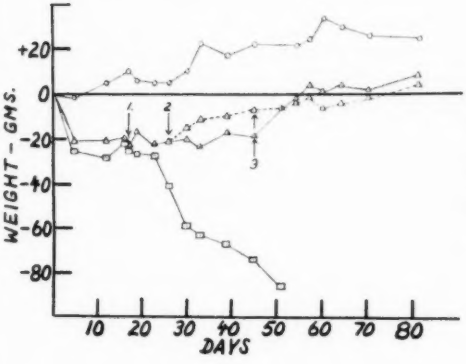

Fig. 1

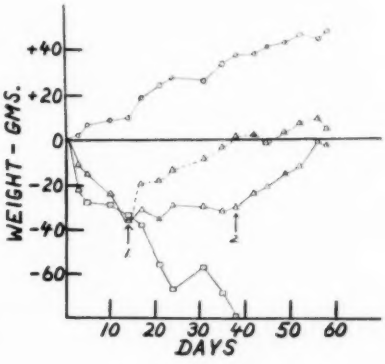

Fig. 2

Fig. 1. Average change in weight of the rats in experiment 1. ⊙——⊙ Group 1. ⊡——⊡ Group 2. △——△ Group 3. △------△ Group 3A. Supplements added at points indicated: 1, group 3 received 500 gamma vitamin B_1 per day; 2, group 3 divided, group 3A given yeast concentrate; 3, groups 3 and 3A fed yeast no. 17000.

Fig. 2. Average change in weight of the rats in experiment 2. ⊙——⊙ Group 1. ⊡——⊡ Group 2. △——△ Group 3. △------△ Group 3A. Supplements added at points indicated: 1, group 3 received 500 gamma of vitamin B_1 per day and group 3A received 500 gamma vitamin B_1 plus 5 per cent yeast concentrate in the diet; 2, group 3A fed yeast no. 17000.

On the 45th day each rat in groups 3 and 3A received, in addition to their above diets, 2 grams of yeast no. 17000 per day. This yeast is rich in the vitamin B_2 complex and low in vitamin B_1. The rats in group 3 made an immediate gain in weight when yeast no. 17000 was added to the diet. Group 3A did not gain weight at an increased rate when yeast no. 17000 was added to the diet, showing that the yeast concentrate (no. 15549) supplied an optimum amount of the vitamin B_2 complex. Every rat in groups 3 and 3A, except one, overcame the effect of the thyroid feeding while still receiving thyroid gland, and regained their lost weight when injected with vitamin B_1 and fed a rich source of the vitamin B_2 complex.

The effect of yeast no. 17000 is not due to its added vitamin B_1 content,

which is very small, for the injection of 500 gamma of vitamin B_1 alone is much above the optimum requirement for vitamin B_1. Leong (11) obtained a maximum storage of vitamin B_1 with a daily oral intake of 30 I.U. of vitamin B_1. Light et al. (12) made a study of the excretion equilibrium of vitamin B_1 in rats and concluded that little or no further storage of vitamin B_1 would take place when the animal had reached an oral intake of between 40 and 65 gamma of vitamin B_1 per day. The use of yeast 17000 also shows that the effect of yeast concentrate no. 15549, which contains 55 I.U. of vitamin B_1 per gram, is not due to its vitamin B_1 content, for the same gain in weight was made with yeast no. 17000.

It was shown (5) that male hyperthyroid rats would not respond to vitamin B_1 and yeast concentrate and gain weight when receiving 100 mgm. of thyroid gland per day. An experiment similar to experiment 1 was therefore performed with male rats receiving 50 mgm. of thyroid gland per day.

Experiment 2. The male rats, weighing about 300 grams, all received diet 8 ad libitum. The groups consisted of: positive controls (group 1), 5 rats; negative controls (group 2), 5 rats; test rats (group 3), 13 rats. As in experiment 1, group 3 was later divided into two groups—group 3 and group 3A. The average weight change for the groups is shown in figure 2. The rats were all gaining weight before the experiment. Groups 2 and 3 were then fed 50 mgm. of thyroid gland per day and showed a rapid loss of weight. On the 14th day group 3 was divided into two groups. Group 3 received a daily injection of 500 gamma of vitamin B_1 and the animals in group 3A also received 500 gamma of vitamin B_1 per day, plus the addition of 5 per cent of yeast concentrate no. 15549 to diet 8. Group 3A began to regain its lost weight while still receiving thyroid gland. Group 3, receiving only the vitamin B_1 supplement, did not lose any more weight and did not regain its lost weight. On the 38th day each rat in group 3 received an added supplement of 2 grams of yeast no. 17000 per day and also began to regain its weight. The results are similar to those obtained in experiment one, and the discussion of experiment one also applies to this experiment.

Food intake. It is known that vitamin B_1 plays an important rôle in influencing the amount of food consumed. Funk and Funk (8) found that nicotinic acid and nicotinic acid amide may also stimulate food consumption. The relation of food intake to the effect of vitamin B_1 and yeast on experimental hyperthyroidism was therefore studied.

All of the rats received diet 8 ad libitum with 200 mgm. of yeast no. 15190 per day. The hyperthyroid rats were treated with vitamin B_1 and yeast concentrate, and the amount of food consumed was measured. The control rats on diet 8 ate an average of 11.6 grams of food per day. Rats on the same diet plus 100 mgm. of thyroid gland per day showed an

initial increase in food intake, averaging 18.3 grams per day which dropped after 28 days to an average of 12.05 grams per day. Rats receiving diet 8 plus 100 mgm. of thyroid gland per day were injected with 500 gamma of vitamin B_1 per day and the average food intake remained at 18.0 grams per day, nearly twice the normal food intake. The addition of 5 per cent yeast concentrate no. 15549 did not further increase the food consumption.

The relationship between the effect of vitamin B_1 and yeast on experimental hyperthyroidism was further studied by limiting the hyperthyroid rats to a definite amount of food per day.

Experiment 3. The rats used in this experiment were transferred to diet 5 and each rat received 13 grams of diet 5 per day plus water ad libitum. After a period of 16 days on diet 5 the rats were divided into 3 groups. Group 1 (positive controls) consisted of 10 rats (5♂, 5♀). Group 2 (negative controls) consisted of 9 rats (5♂, 4♀). Group 3 (test rats) consisted of 19 rats (9♂, 10♀). On the 17th day each rat in groups 2 and 3 also received 100 mgm. of thyroid gland per day.

Group 1 gained weight on the basal diet whereas groups 2 and 3 lost weight on the same diet when thyroid gland was fed. After the thyroid gland had been administered for 8 days, at which time the females in group 3 had lost from 10 to 30 grams and the males had lost from 35 to 62 grams in weight, 500 gamma of crystalline vitamin B_1 were injected per day in each animal in group 3. At the same time the yeast content of the diet of one-half of the test rats in group 3 was raised to 10 per cent (diet 6). This was to determine if an increased amount of the vitamin B_2 complex in the form of yeast, would have any effect on the influence of the vitamin B_1 on the loss of weight of the hyperthyroid rats.

The 500 gamma of vitamin B_1 enabled some of the rats in group 3 to gain weight for 2 to 3 days following the first injection of vitamin B_1. On the following days, however, the rats continued to lose weight. The negative controls, receiving 100 mgm. of thyroid gland, were steadily losing weight and the positive controls continued to gain weight. The injection of the vitamin B_1 was continued for 12 days during which time three of the negative controls and five of the test rats died, the test rats dying about 2 days after the negative controls. The 500 gamma of vitamin B_1 administered for 12 days to rats with 5 per cent or 10 per cent yeast in their diet did not stop the loss of weight and did not influence the number of deaths of the hyperthyroid rats *when the rats were limited to 13 grams of food per day.*

Estrual cycle. It has been found that thyroid feeding will produce complete diestrus, or in certain dosages will lengthen the diestrous period (9, 16). Studies of the estrual cycle of hyperthyroid rats were therefore made to see if the vitamin B_1 and yeast concentrate treatment would also enable the estrual cycle of the hyperthyroid rats to return to normal

while the rats were still receiving thyroid gland. Studies were also made on the effect of vitamin B_1 on the estrual cycle of normal rats.

The estrual cycle studies were made on the rats used in experiment one, and the group numbers used refer to the groups in that experiment. Control smears, which were taken on all rats for 12 days before the thyroid feeding started, showed that all of the rats had normal estrual cycles. Group 1 (positive controls) maintained normal estrual cycles, averaging 4.77 days, throughout the experiment, and therefore will not be shown in figure 3. As soon as the thyroid feeding was started group 2 exhibited a few normal cycles and then went into a continuous diestrus (fig. 3). Groups 3 and 3A also showed a few normal cycles, after the thyroid feeding was started, and then began to go into diestrus. As the animals were

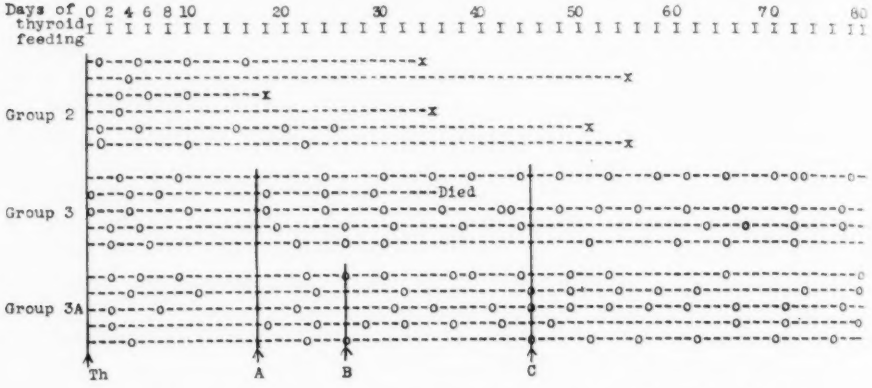

Fig. 3. Estrual cycle of rats; *o*, marks time of estrus (stage 2-3); (—) diestrus; *x*, rat dissected. *Th*, thyroid feeding started. *A*, vitamin B_1 injections begun. *B*, yeast concentrate fed. *C*, dried yeast no. 17000 fed.

rapidly losing weight (cf. fig. 1), 500 gamma of vitamin B_1 were injected daily, starting on the 17th day, and the estrual cycles began to reappear almost immediately. It should be noted that group 3 remained practically constant in weight, when vitamin B_1 was injected, between the 17th and 45th days, and yet typical estrous periods were obtained while the rats were still receiving thyroid gland. When the various yeast supplements were added to the diet of the hyperthyroid rats, the rats began to gain weight and the normal estrual cycles continued. Various longer periods of diestrus occurred in some of the rats, but on the whole the cycles are quite regular. The average length of the estrual cycle of the test rats, from the 17th to the 80th day, was 6.54 days for group 3 and 6.65 days for group 3A, this average being slightly higher than the average for group 1.

As a control 9 normal, adult female rats, receiving diet 9 ad libitum, were

given daily subcutaneous injections of 500 gamma of vitamin B_1 for 44 days. The rats exhibited normal cycles, averaging 4.70 days, showing that 500 gamma of vitamin B_1, injected daily for 44 days, do not influence the estrual cycle of normal rats.

It is evident that the vitamin B_1 and the vitamin B_2 complex, besides enabling the rats to gain weight, will also enable hyperthyroid rats, which are entering into diestrus, to maintain a normal estrous cycle.

Discussion. Vitamin B_1 and some as yet unidentified component or components in yeast, probably part of the vitamin B_2 complex, exert a remarkable effect on the weight of rats receiving thyroid gland. Rats which have lost weight will regain their lost weight when injected with vitamin B_1 and fed a rich source of the B_2 complex, while still receiving thyroid gland. Vitamin B_1 injections alone will enable the rats to stop losing weight, but the rats will not begin to regain their lost weight, with the diets used, until yeast or a yeast concentrate is also fed. This demonstrates that during experimental hyperthyroidism there is a deficiency of the vitamins of the B_2 complex, or a requirement greater than normal. This increased requirement may or may not be influenced solely by the heightened metabolism, but may be connected more intimately with thyroid function. Cowgill and Dann (2) failed to influence the vitamin B_1 requirement by feeding dinitrophenol, whereas this requirement can be easily influenced by thyroid feeding.

In studying the prevention of experimental hyperthyroidism Sure and Buchanan (14) found that better results were obtained when a large amount of autoclaved yeast was included in the basal diet. Himwich, Goldfarb, and Cowgill (10) regarded the effect of the Harris yeast which they used as due mainly to its vitamin B_1 content. Their results must now be interpreted to include the effect of the vitamin B_2 complex that is present in yeast.

Other vitamin deficiencies may also be present during experimental hyperthyroidism. Sure and Buchanan (15) noted the development of xerophthalmia in hyperthyroid rats. We also found in preliminary experiments on hyperthyroid rats that xerophthalmia would develop when the diet contained 2 per cent cod liver oil (U.S.P.). When the cod liver oil content was raised to 4 per cent (diet 8) none of the test rats developed xerophthalmia. Vitamin A or carotene, when administered to rats receiving small amounts of thyroxin, will retard the loss of weight (13, 15, and others) but will not *prevent* the loss of weight. However, in all experiments on hyperthyroidism an abundance of vitamin A should be provided so that no deficiency of vitamin A will act as a limiting factor in studying weight gain.

It was observed that hyperthyroid female rats will respond to vitamin B_1 and yeast treatment, as judged by the gain in weight, at a higher level of

thyroid feeding than will male rats fed thyroid gland. Male rats fed the same amount of thyroid gland as female rats will also lose weight at a faster rate. It would seem, therefore, that the reason for the sex difference in response will be found in the different sex hormones possessed by the male and female rat, and that some of the female sex hormones may have some antithyrogenic action. Several reports have already appeared on the antithyrogenic effect of the sex hormones. Bodansky and Duff (1) and Danforth and Loumos (4) have also shown that thyroid is less effective when fed to pregnant animals.

Undifferentiated vitamin B_1 (10), and crystalline vitamin B_1, as reported above, were found to increase the food intake of hyperthyroid animals. This is most important in counteracting the rapid catabolism produced by thyroid feeding. The antithyrogenic action, however, is not due to an increased calorie intake alone, as the other vitamins of the B_2 complex must be provided, as in the form of yeast, for vitamin B_1 will stimulate food intake but the rats will not regain their lost weight under the conditions used until the vitamin B_2 complex is also fed. The body stores of vitamin B_1 are also reduced during experimental hyperthyroidism (6).

The rôle that vitamin B_1 and the vitamin B_2 complex may play in the treatment of human hyperthyroidism is as yet unknown. Their action may be limited, since Elmer et al. (7) have found, using guinea pigs, that vitamin B_1 is not antagonistic to thyroid stimulation with the thyreotropic principle of the anterior pituitary. The effect of yeast under such conditions has not as yet been studied. From the above evidence, however, vitamin B_1 and yeast may be of value in preventing the loss of weight accompanying human hyperthyroidism.

SUMMARY AND CONCLUSIONS

1. Vitamin B_1 and yeast will enable hyperthyroid rats which have lost weight to regain their lost weight while still receiving thyroid gland.

2. Vitamin B_1 and yeast concentrate greatly stimulated the food intake of hyperthyroid rats. This is an important factor in enabling rats receiving thyroid gland to gain weight, but it is not the only factor, for although vitamin B_1 will stimulate the food intake the rats will not gain weight under the conditions used until a rich source of the vitamin B_2 complex is added to the diet.

3. Hyperthyroid rats limited to 13 grams of food will not stop losing weight when vitamin B_1 and yeast are administered.

4. The vitamin B_1 and yeast treatment will also enable hyperthyroid rats that are entering into diestrus to maintain a normal estrous cycle, averaging 6.54 days.

5. The estrual cycle of normal rats is not influenced by the daily injection of 500 gamma of vitamin B_1 for 44 days.

The authors wish to thank Dr. Ralph H. Cheney of Long Island University for his interest and counsel in this work, and Mr. R. F. Light of The Fleischmann Laboratories for his interest and valuable advice.

REFERENCES

(1) Bodansky, M. and V. B. Duff. Endocrinology **20**: 537, 1936.
(2) Cowgill, G. R. and M. Dann. Yale J. Biol. and Med. **8**: 501, 1936.
(3) Cowgill, G. R. and M. L. Palmieri. This Journal **105**: 146, 1933.
(4) Danforth, D. N. and S. Loumos. Proc. Soc. Exper. Biol. and Med. **34**: 870, 1936.
(5) Drill, V. A. Proc. Soc. Exper. Biol. and Med. In press.
(6) Drill, V. A. This Journal **122**: 486, 1938.
(7) Elmer, A. W., B. Giedosz and M. Scheps. C. R. Soc. Biol. **126**: 1037, 1937.
(8) Funk, C. and I. C. Funk. J. Biol. Chem. **119**: xxxv, 1937.
(9) Halpern, S. R. and I. E. Hendryson. Proc. Soc. Exper. Biol. and Med. **33**: 263, 1935.
(10) Himwich, H. E., W. Goldfarb and G. R. Cowgill. This Journal **99**: 689, 1932.
(11) Leong, P. C. Biochem. J. **31**: 367, 1937.
(12) Light, R. F., A. Schultz, L. Atkin and L. Cracas. J. Nutrition. In press.
(13) Logaras, G. and J. C. Drummond. Biochem. J. **32**: 964, 1937.
(14) Sure, B. and K. S. Buchanan. J. Nutrition **13**: 513, 1937.
(15) Sure, B. and K. S. Buchanan. J. Nutrition **13**: 521, 1937.
(16) Weichert, C. K. and R. W. Boyd. Anat. Rec. **58**: 55, 1933.

THE REACTIONS OF THE CLOTHED HUMAN BODY TO VARIATIONS IN ATMOSPHERIC HUMIDITY

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In a recent paper (Gagge, Winslow and Herrington, 1938), we have presented data on the reactions of the clothed human body to various combinations of air and wall temperature, with a relative humidity of 40 to 50 per cent and a standard turbulent air velocity of approximately 5 meters per minute. The present paper deals with experiments in which air and wall temperatures were identical with each other but were varied over a wide range, with simultaneous variation in relative humidity.

The material available consists of data from 124 experiments conducted with each of two subjects (nos. IX and X), during the summer of 1937, and 20 supplementary experiments (17, 18, 1-A, and 3-A) conducted with subject IX in the spring of 1938. The primary studies were made under 16 different conditions of air (and wall) temperatures ranging from 18° to 37°C., and with two different relative humidities at each level of air temperature. "Low humidities" ranged from 24 to 35 per cent, "high humidities" from 75 to 88 per cent. The "low humidity" series was extended by conditions 17 and 18 to an air temperature of 42.5°C., and conditions 1-A and 3-A repeated with an even lower humidity (12–17 per cent) conditions 1 and 3 of the primary series.

The physical characteristics of subjects IX and X are given in table 1. The general results for the 20 experimental conditions are presented in table 2. In the primary series, the data for conditions 1–15 represent averaged values for subjects IX and X together. The data for condition 16 and for the additional experiments 17, 18, 1-A, and 3-A, represent averages for subject IX alone.

All other conditions of the experiments were identical with those described in our earlier communication.

Influence of relative humidity in the cold zone. As pointed out in our previous contribution (Gagge, Winslow, and Herrington, 1938), an environmental temperature of 24.5°–25.5°C. marks the upper limit of the zone of body cooling. In the area below this point, as indicated in table 2 and in figures 1 and 2, the influence of moderately high, as compared with

TABLE 1

Physical constants of subjects when clothed

SUBJECT	HEIGHT	WEIGHT	DUBOIS LINEAR AREA	EFFECTIVE RADIATION AREA	CONSTANT FOR HEAT LOSS BY CONVECTION (FOR TURBULENT AIR VELOCITY 5 M/MIN.)
	meters	*kgm.*	*sq. m.*	*sq. m.*	
IX	1.72	62.5	1.60	1.05	4.68
X	1.78	71.0	1.81	1.41	5.94

TABLE 2

Data—humidity study—clothed

GROUP	T_A	RH	T_B	T_S	T_{Cl}	$\Delta T_B/\Delta t$	M	E	$R+C$	S	w_μ
	°C.	per cent	°C.	°C.	°C.	°C./hr.	kg. cal./m²/hr.	kg. cal./m²/hr.	kg. cal./m²/hr.	kg. cal./m²/hr.	
1	18.4	35	36.7	31.4	27.9	−0.16	43	−13	−66	34	4
2	20.9	26	36.8	32.4	30.1	−0.14	47	−14	−64	31	4
3	23.9	29	36.8	33.8	32.1	−0.10	46	−16	−57	27	5
4	26.8	24	37.1	34.7	33.7	000	48	−19	−48	19	5
5	29.3	24	37.2	34.9	34.8	000	50	−32	−39	21	9
6	32.2	25	37.1	35.2	34.9	000	43	−40	−19	11	12
7	35.0	30	37.3	35.8	36.5	−0.02	52	−52	−11	11	16
8	36.8	33	37.2	36.2	36.8	+0.04	46	−59	0	13	20
17	39.8	31	36.9	35.9	38.3	+0.06	51	−63	11	1	23
18	42.5	24	37.3	36.7	39.3	+0.34	55	−86	22	9	29
9	18.8	75	36.8	31.9	29.2	−0.25	45	−9	−73	37	4
10	21.6	77	36.9	33.5	30.9	−0.08	46	−10	−66	30	4
11	24.1	79	36.7	33.8	31.9	−0.06	45	−13	−55	23	6
12	26.9	81	36.8	34.8	33.2	−0.06	45	−20	−44	19	10
13	29.7	87	37.4	35.2	34.4	000	49	−29	−33	13	19
14	32.3	88	37.2	35.5	35.4	+0.03	48	−35	−22	9	30
15	34.2	86	37.4	35.7	36.4	+0.28	51	−38	−16	3	46
16	36.8	76	37.8	37.9	37.6	+1.0	48	−49	−5	6	35
1-A	18.4	17	36.3	32.4	28.7		48	−16	−72	40	5
3-A	23.9	12	36.5	34.2	31.9		44	−17	−56	29	4

Conditions 1–15 represent averaged data for subjects IX and X combined. Conditions 16, 1-A, and 3-A represent mean data for subject IX alone.

T_A, air temperature in °C.; RH, relative humidity, as a percentage; T_B, rectal temperature in °C.; $\Delta T_B/\Delta t$, rate of change of rectal temperature in °C./hr.; T_S, mean skin temperature in °C.; T_{Cl}, mean exposed skin-clothing surface temperature in °C.; M, the metabolism in kgm. cal./m²/hr.; E, the evaporation loss in kgm. cal./m²/hr.; $R + C$, total loss by radiation plus convection to the environment in kgm. cal./m²/hr.; S, storage, or the rate of cooling (when positive) or heating (when negative) of the body; w_μ, an index measuring the extent of wettedness of the moist surfaces of the body, expressed in kgm. cal./m²/hr./cm. of Hg of vapor-pressure difference.

low relative humidity (comparing a relative humidity of about 80 per cent with one of about 30 per cent), is very slight. In this cold region, the total evaporation loss is minimal and insensible, and, as would be expected, it is slightly lower for the high humidities (see fig. 2). Skin temperature tends to be a trifle higher for high humidity (fig. 1) and heat loss by radiation plus convection a trifle greater, but these differences are slight. Metab-

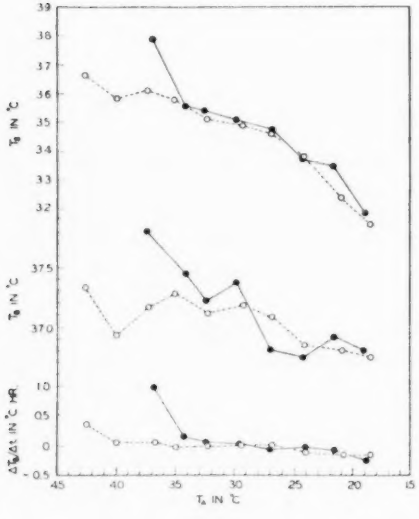

Fig. 1

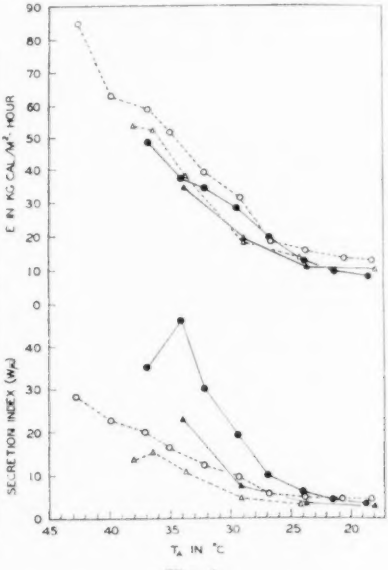

Fig. 2

Fig. 1. Relation of skin temperature (T_S), rectal temperature (T_B), and the time rate of change of rectal temperature ($\Delta T_B/\Delta t$) to dry bulb temperature (T_A) for the clothed subjects; open circles for low humidity, closed circles for high humidity.

Fig. 2. Relation of total evaporation (E) secretion index (w_μ) to dry bulb temperature (T_A) for both clothed subjects (circles) and nude subjects (triangles); open circles and open triangles for low humidity, closed circles and closed triangles for high humidity.

olism, body temperature, and storage are essentially the same with either high or low relative humidity.

The possible influence of a still drier atmosphere is demonstrated by comparing condition 1-A with condition 1 at 18.4°C., and condition 3-A with condition 3 at 23.9°C. For an air temperature of 23.9°, the results for condition 3-A at a humidity of 12 per cent were essentially identical with those observed for condition 3 at a relative humidity of 29 per cent, while even at the very low air temperature of under 19°, the water loss for

low relative humidity was only 22 per cent greater. The actual differences in evaporative heat loss and in the corresponding amounts of moisture loss, for all three humidities, observed at each of two operative temperatures, were as follows:

AIR TEMPERATURE	RELATIVE HUMIDITY	EVAPORATIVE HEAT LOSS	CORRESPONDING WATER LOSS
		kgm./sq.m./hr.	*gm./hr.*
18.4°–18.8°	17	16	28
	35	13	23
	75	9	16
23.9°	12	17	30
	29	16	28
	79	13	23

These results are about what might be expected from a computation of vapor pressure differences governing the evaporative process and they tend to confirm an earlier suggestion of ours (Gagge, 1937; and Winslow, Herrington, and Gagge, 1937a) that the moist surfaces governing insensible perspiration reach an approximately constant minimal area at low operative temperatures.

It will be noted that the decreased heat loss by evaporation attained by raising the relative humidity of the air from 29–35 per cent to 75–79 per cent is only 4 kilogram-calories/m^2./hr. This is equivalent to raising the air temperature about 0.5°C., provided the surface clothing temperature remains constant.

Influence of relative humidity in the hot zone. At temperatures above 28.5°–29.5°C., the clothed body is in the zone of evaporative regulation and, here, the relative humidity of the atmosphere has highly significant effects.

The most striking of these effects are indicated in figure 2, where we have included, for comparison, earlier results obtained with nude subjects (see table 2, Winslow, Herrington, and Gagge, 1937a).

It will be noted that wetted area, or the index of sweat secretion, as originally defined by one of us (Gagge, 1937) in terms of (w_μ), shows a definite and sharp increase with rising temperature for all conditions. This increase begins at 28.5° for nude subjects and at about the same point for clothed subjects with low relative humidity. For clothed subjects with high relative humidity, it begins at a lower air temperature (23.9°). For both clothed and nude subjects, the rise in wetted area for increasing air temperatures is very much steeper for high relative humidity conditions.

It is clear from the upper graph of figure 2 that, with the clothed subject, evaporative heat loss begins to increase slightly at an air temperature of 22° and rises more sharply above 26.5°. At about 24° the wetted area

increases to balance the decreasing calorie demand and rises more sharply with high than with low relative humidity. From this point, the increase in wetted area is enough greater with high relative humidity than with low relative humidity to nearly balance the lower moisture demand of the environment. Yet, in spite of the rapid and marked increase in wetted area, evaporative heat loss for high humidity falls slightly below that for low humidity.

With high relative humidity, in the hot zone, skin temperature and body temperature rise sharply (see fig. 1), metabolism tends to be greater, storage (cooling) is less, wetted area increases greatly and discomfort becomes pronounced. The lower wetted area at 36.8° as compared with 34.2° was associated with a slightly lower atmospheric humidity. Yet in these experiments at 36.8°C., with 76 per cent relative humidity, table 2 shows a body temperature of 37.8° and a mean skin temperature of 37.9°C. These data are for subject IX. Subject X, under similar conditions, was nauseated and his subjective symptoms were so severe that the experiment had to be discontinued.

With low relative humidity, on the other hand, the rise in wetted area is much more gradual and the evaporative heat loss greater than with high relative humidity (fig. 2). Skin temperature does not exceed 36.2° and rectal temperature does not exceed 37.3° until air temperature reaches 42.5°. Metabolism also rises only at 42.5°, although maximum discomfort begins to be experienced at 39.8°. At 42.5°, although the body was still in reasonably good thermal equilibrium, the discomfort of the subject was so great that no more extreme conditions could be used.

The most striking index of failure of the evaporative process of heat regulation is indicated in the lower graph of figure 1. This represents the rate of increase in rectal temperature expressed in degrees Centigrade per hour. It will be seen that, with high atmospheric humidity, this graph breaks sharply above a T_A of 34° while with low atmospheric humidity the break comes only at 40°C., and is even then much less sharp.

Physiological mechanism governing sweat secretion. In order to gain some idea of the factors which may govern sweat secretion, we have plotted in figure 3, wetted area or index of secretion against rectal temperature (T_R) and against mean skin temperature (T_S) for our clothed subjects. It will be noted that, below a T_S of 34°, secretion is minimal. Between 34° and 34.5° it rises slowly and above 35°, sharply (but more sharply with high than with low humidity). Graphs for regional skin temperatures (not reproduced here) show that the sharp rise in secretion corresponds with the following critical skin temperatures: head, 35°; upper extremities, 34.4°–35.3°; trunk, 34.4°–35.3°; lower extremities, 33.9°.

Clearly, the different reactions of sweat secretion under conditions of high and low humidity are not governed by changes in skin temperature

alone. The upper graph of figure 3 suggests that body temperature may play an important part, since all the very high values for the secretion index are associated with rectal temperatures of 37.2° and over; and since here the graphs for high and low humidity follow much the same course.

Significance of wetted area. In the present paper we have so far expressed Wetted Area, or index of secretion, in its basic units of kilogram-calories of heat loss per square meter per hour per centimeter of Hg vapor-pressure difference between the vapor pressure of the skin and that of the ambient air. This is the factor we have earlier described as (w_μ). In our previous papers we have also expressed Wetted Area as a percentage of

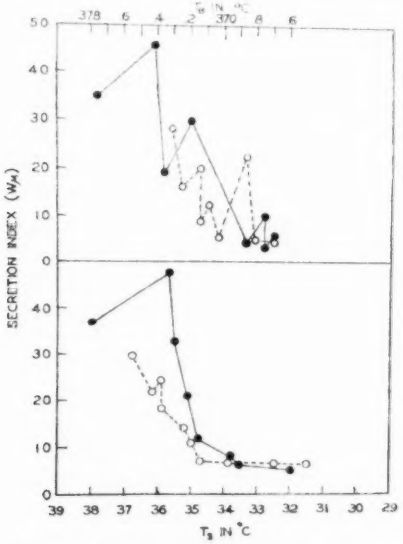

Fig. 3. Relation of secretion index (w_μ) to rectal temperature (T_B) and skin temperature (T_S) for clothed subjects; open circles for low humidity, closed circles for high humidity.

the maximum value attained, as experimentally determined, at the point where evaporation fails and sweat begins to run off the body without exerting its full evaporative cooling power. For the nude body we found this maximum value of (w_μ) to be 29.9 kilogram-calories per square meter of body surface per hour per centimeter of Hg vapor-pressure difference (Gagge, 1937); and in previous papers on nude subjects we expressed Wetted Area in terms of a percentage of this figure. In order to avoid unnecessary assumptions we are using the actually observed (w_μ) in the present communication.

For our data with the clothed subject and with high relative humidity,

we find a value of ($w\mu$) at 32.3°C., of 30 and at 34.2°C., of 46. It is clear, however, that at 34.2° the full effect of evaporative cooling is not being exerted, since actual evaporative heat loss remains about the same as at 32.3° (38 kilogram-calories as compared with 35), while both skin temperature and body temperature are going up. It is apparent that where much more than 30 kilogram-calories per square meter of body surface per hour per centimeter of Hg vapor-pressure difference is lost, a considerable part of the evaporation must take place from the clothing surfaces, and in such a way that the full corresponding cooling effect is not attained.

Missenard (1934), in a valuable analysis of the physical principles underlying convective and evaporative heat loss, gives formulae which should lead to considerably higher evaporative heat losses than this. With the human body, either clothed or unclothed, our direct observations seem to indicate that the figure of approximately 30 kilogram-calories represents the maximum of actually effective cooling which can be attained.

Another question of much interest[1] concerns the significance of the increase observed by us in Wetted Area with increasing operative temperature and increasing relative humidity. When the body evaporates the same amount of moisture with an atmospheric relative humidity of 60 per cent as with one of 30 per cent (at the same air temperature), there must obviously be an increase in Wetted Area to bring about this result. We have assumed that this increase in Wetted Area is due to an increased activity of sweat glands. The question has been raised whether any such assumption is necessary, since, with the same amount of total evaporation, the observed results might be explained on purely physical grounds, by the spreading of the sweat secreted by each gland over a wider area about the gland. If all the sweat glands secreted continuously at a fixed rate, this explanation would be adequate, and indeed, would be the only one admissible. An increase in Wetted Area with a constant total evaporation is possible *either* through bringing a larger number of sweat glands into activity at a lower mean rate of secretion, *or* through a wider spread over the skin surface of the moisture discharged by the originally active glands. The first process is *physiological*, the second *physical*. The question as to which process is involved depends upon 1, the mode of action of individual glands maintaining a thermal equilibrium, and 2, the thermal threshold of the skin.

It is well known by direct observation of individual pores, and by measurements of total evaporation from small skin fields, that glands secrete by quanta and not continuously, that the individual rate is variable, and that the total rate from a small field oscillates around the steady rate for the body as a whole. This means that sensible loss, as contrasted with

[1] Raised in correspondence with Prof. E. B. Phelps of Columbia University.

insensible minimum perspiration, is an active process, neurally regulated, in which an apparent constant rate of evaporation is maintained by a moment-to-moment change in the population of active glands. These must exhibit in their individual action, threshold, latency, adaptation, and the general properties we associate with neuro-motor units. With this physiological view of the mode of action of the sweat glands in mind (Jürgensen, 1924, 1925, 1927, 1929), we may examine the second question.

The problem as to how a constant rate of evaporation is maintained at equilibrium is quite as important as the question how adaptation is made to a change of humidity when sensible heat of the environment remains constant. There should, however, be no essential difference between these two processes. Although the mean skin temperature remains constant at equilibrium, the over- and under-shooting around the mean of the evaporative loss from small skin fields constituted an active "hunting" process in which over-heating and over-cooling of skin fields modulates central stimulation as well as local threshold. The only essential change on increase of relative humidity is a transitory predominance of fields which are, at the moment, over-heating.

The main point in question is the thermal sensitivity of the skin. This has been shown to be of the order of 5×10^{-4} gm. cal./sec/cm^2., or 18 kgm. cal./m^2/hr. (Hardy and Oppel, 1936). In practice, this means that a rise of 0.003°C., at a rate of 0.001° per second is an adequate stimulus. We may thus determine how large a change in the skin temperature is needed to exceed such a threshold.

As an example, we may take the following; Subject, weight 55 kgm. Total metabolism, 65 kgm. cal./hr. Evaporation, 55 kgm. cal./hr. Air temperature, 33.2°C. Skin temperature, 35.4°C. Relative humidity, 20 per cent. Let us postulate a sudden increase in relative humidity to 80 per cent, such as would physically reduce the rate of evaporation loss to 35 kgm. cal./hr. The skin temperature will instantly begin to traverse a rising curve, whose initial section would be identical with that followed if a change in air temperature were wholly responsible for the new equilibrium. This condition, on the basis of physical heating curves, would be reached in 13 minutes, and assuming, for simplicity, that the change is linear, the skin temperature increment in the first second would be 0.0017°C. This approximation is nearly twice the threshold value and, since the curve is actually logarithmic, the actual value would be much higher. Since sweat secretion is so closely related to skin temperature (fig. 3), we have a situation in which the active regulation of secretion will obviously occur rather than simple physical equilibrium.

Limits to the evaporative process of thermal regulation. In an earlier communication (Winslow, Herrington, and Gagge, 1937a) we derived a formula by which one may compute for the nude subject the upper limits

of air temperature and relative humidity beyond which evaporative regulation is impossible. A similar computation for our clothed subjects may be made as follows:

The exchange of heat with the environment for the human body in thermal equilibrium has been shown to be described by the fundamental relation,

$$M - E = k_o\,(T_{cl} - T_A), \tag{1}$$

where k_o is the environmental constant and T_{cl} is the clothing temperature. If evaporation, E, takes place at the skin surface, then

$$M - E = k_{cl}\,(T_S - T_{cl}), \tag{2}$$

where k_{cl} represents the conductance of the clothes. Eliminating T_{cl} from (1) and (2), and solving for E, we have

$$E = M - \frac{k_o k_{cl}}{k_o + k_{cl}}\,(T_S - T_A). \tag{3}$$

In our earlier communication we showed that the evaporation, E, was also described by equation

$$E = (w\mu)\,[\epsilon(T_S) - RH/100 \times \epsilon(T_A)], \tag{4}$$

where $(w\mu)$ is the wetted area and $\epsilon(T_S)$ and $\epsilon(T_A)$ are the saturated vapor pressures of air at temperatures T_S and T_A. Equating (3) and (4), and solving for RH, the relative humidity, we have

$$RH = 100/\epsilon(T_A)\left[\epsilon(T_S) - \left\{M - \left[\frac{k_o k_{cl}}{k_o + k_{cl}}\right](T_S - T_A)\right\}\right] \Big/ (w\mu). \tag{5}$$

We have seen that the critical value for $(w\mu)$ may be taken as 30. This occurs at a T_A of 32.2° with a skin temperature of 35.6° and a metabolism of 47. A representative value for k_o has been found to be 7.02, while in a previous paper (Gagge, Winslow, and Herrington, 1938) we showed that k_{cl} was 15.8. We have, on substituting all these values in (5),

$$RH = 100/\epsilon(T_A)\,[4.34 - \{47 - 4.9\,(35.6 - T_A)\}]/30,$$

or

$$= 100/\epsilon(T_A)\left[\frac{51.9 - T_A}{6.13}\right]. \tag{6}$$

From this equation we are able to evaluate for any air temperature the relative humidity which will correspond to the upper limit of evaporative cooling. This has been done in the table below, in which corresponding values previously found for the nude subject are also entered. Such values naturally depend on the metabolism of the subject and his posture but

hold for the conditions of our experiments (subject at rest in a semi-reclining position).

Upper limits of evaporative regulation

ENVIRONMENTAL TEMPERATURE	RELATIVE HUMIDITY	
	Clothed subject	Nude subject
52.7	0	
50.0	5	
45.0	18	1
42.5	26	13
40.0	38	29
37.5	51	47
35.0	69	70
32.5	89	100
31.0	100	

It will be noted that below 35°, the nude body can endure a higher relative humidity than the clothed. However, above 35° this relation is reversed; the clothed body is able—with further rise in air temperature—to adapt to higher and higher relative humidities as compared with those which limit the adaptive powers of the nude subject. This is because, at air temperatures above skin temperature, the lower constant (4.9) acting upon the skin surface (as determined by the insulating effect of clothing in the case of the clothed subject) limits the reception of heat from the environment, while evaporative heat loss is as great as with the nude subject and indeed, slightly greater, due to the wick-action of the clothing (see fig. 2). These facts illustrate why the Arabs and other desert people protect themselves with heavy clothing against the dry, but intense, heat of the environment to which they are exposed.

Seasonal adaptation. Our experiments have been conducted at various times of the year; and we felt it important to know how far such results as those discussed above might be influenced by seasonal physiological adaptation. For this purpose we conducted a special series of experiments with two subjects (VII and IX) during the winter and again—under the same experimental conditions—during the summer. Four conditions (varying in air temperature) were observed at each season with the subjects nude and five with the subjects clothed. Air movement was always minimal (5 meters per minute), relative humidity was moderate (averaging 51 per cent in winter; 59 per cent in summer), and air and walls were at the same temperature. Air temperatures were varied from 17° to 36°C.

Careful analysis of the data showed no significant differences between winter and summer reactions to a given air temperature, for either subject or for either nude or clothed condition, with respect to metabolism, skin temperature, rectal temperature, or skin conductance. On the other

hand, evaporative heat loss and secretion index were slightly, but definitely, greater (under warm experimental conditions) during the summer than during the winter—as indicated in figure 4. It appears, as would be expected, that the sweat-secreting mechanism is in more effective working order during the summer season, when the body is acclimated to the task of dealing with high atmospheric temperatures.

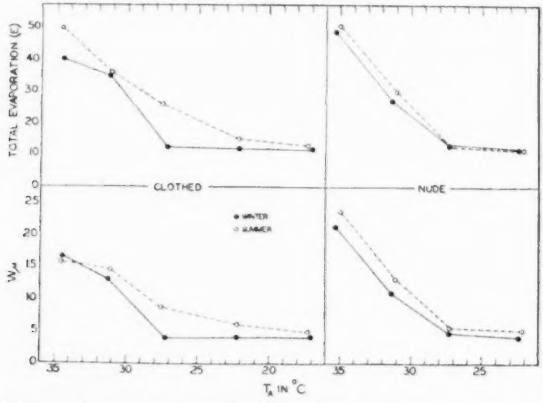

Fig. 4. Relation of total evaporative heat loss and secretion index (w_μ) to air temperature for clothed and nude subjects during the winter and summer seasons.

CONCLUSIONS

From our studies of the influence of atmospheric relative humidity (between 12 and 88 per cent) upon the thermal reactions of clothed subjects, we may draw the following conclusions.

1. In the zone of body cooling (below an environmental temperature of 25° ± 0.5°C.) sweat secretion and wetted area are minimal. In this area, the relative humidity of the atmosphere has a scarcely appreciable influence upon physiological reactions in general. With a relative humidity of 30–35 per cent, the evaporative heat loss is about 4 kilogram-calories per square meter per hour less than with a relative humidity of 75–80 per cent. This is the equivalent of a rise in air temperature of about 0.5°.

2. When environmental temperature exceeds 25°C., the clothed body begins to adapt to the lowered calorie-demand of the environment by an increase in wetted area. This increase is much sharper with high relative humidity than with low relative humidity and up to 32° ± 0.3°C., the body maintains a reasonable adaptation to decreases in calorie demand. This process we attribute to the stimulation of increasing numbers of sweat glands rather than to any purely physical process of spreading of water

over the skin. The stimulation involved cannot be accounted for by rise in skin temperature alone, but is apparently also directly influenced by increasing internal body temperatures.

3. The upper limit of evaporative regulation is reached—for both nude and clothed subjects—at the point where about 30 kilogram-calories per square meter per hour is lost per centimeter of Hg vapor-pressure difference. This point is attained for the nude subject, with minimum relative humidity, at a little over 43°C. The clothed subject, with greater protection furnished by the clothing against heat inflow from the environment, and equal (or greater) evaporation, can regulate up to nearly 52°C., with minimum relative humidity. With very high relative humidity, however, (70–80 per cent) the clothed subject adjusts less well than the nude subject. Adaptation fails under such conditions at an operative temperature below 35°C.

4. During the summer season, the mechanism of sweat secretion is in better working order than in winter so that the body responds to a given condition (in the zone of evaporative regulation) with a somewhat higher secretory activity. Other physiological responses show no significant seasonal differences.

REFERENCES

GAGGE, A. P. This Journal **120:** 277, 1937.

GAGGE, A. P., C.-E.A. WINSLOW AND L. P. HERRINGTON. This Journal **124:** 30, 1938.

HARDY, J. D. AND T. W. OPPEL. Physics **7:** 466, 1936.

HOUGHTEN, F. C. AND C. P. YAGLOGLOU. A. S. H. V. E., Trans. **29:** 163, 1923a.
A. S. H. V. E., Trans. **29:** 361, 1923b.

JÜRGENSON, E. Deutsch. Arch. f. klin. Med. **144:** 193; **144:** 248, 1924.
Deutsch. Arch. f. klin. Med. **149:** 157, 1925.
Deutsch. Arch. f. klin. Med. **155:** 342, 1927.
Deutsch. Arch. f. klin. Med. **165:** 165, 1929.

MISSENARD, A. Extrait de *Chaleur* et *Industrie* June, 1934.

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **120:** 288, 1937a.
Am. J. Hyg. **26:** 103, 1937b.

THE MINUTE-VOLUME UPTAKE AND OUTPUT OF SUBSTANCES PERFUSED THROUGH LIVER SURVIVING IN AN ONCOMETER

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The exchange of substances between organs and the general circulation has been the object of many experiments, the authors of which have been content with knowledge of mere concentration of the substances at the sites of inflow and outflow of the organ. But in the final analysis it is *the differences in uptake and output per unit of time*, quite as much as differences in mere concentration at the sites of inflow and outflow, which determine the rôle played by an organ in the general body economy.

In chronic cases where one factor acts upon an organ for a relatively long period of time, the analyses of slices of the organ at start and finish have furnished satisfactory evidence of loss and gain. But if one wishes to know the effects of a short lasting stimulus, or of a single, small dose of a drug, during successive brief periods of time, then the frequent removal of slices becomes not only quite impracticable but also fatal to the object of the experiment.

The present paper deals with the results of the analyses of the outflow of turtles' livers surviving in an oncometer. The outflow was collected in successive samples of approximately equal volumes and at relatively short time-intervals. The manner of construction and application of the oncometer, and methods of isolation, perfusion and mounting the liver-preparation in the oncometer have been described by Snyder, 1938.

While the results here reported deal with the uptake and output of substances by the organ-preparation in its entirety, the senior author believes that, under certain conditions, the method will permit extension to the determination of the actual uptake and output by the tissue cells of an organ per unit of time. For if there is an indifferent ion present to which the cells are quite permeable under all conditions then the knowledge of its concentration at inflow and outflow, before and after stimulus or the injection of a drug, together with the knowledge of the exact volumes of inflow and outflow over identical time-intervals, will enable one to determine

[1] Henry Strong Denison Scholar for 1938-39.

whether the cells have taken up water or given it off, and finally to evaluate what share vascular changes and what share cellular volume changes play in the total volume changes the organ under observation undergoes.

The procedures observed were as follows: After the establishment of equilibrium between liver-tissues and the perfusing fluid, collection of successive samples at the hepatic outflow was begun and continued for periods (in various experiments) covering from 30 to 90 minutes. The number of samples collected varied accordingly from 6 to 24. The samples were caught (from an automatic tipping vessel at the outflow) in 15 ml. centrifuge tubes; their volumes were at once read off to the tenth ml. from the graduations on the tubes, recorded, and then centrifuged, in order to separate the few cells that continued to be washed out of the liver regardless of the duration of previous perfusion with cell-free perfusing fluids. Fractions of the filtrates were then taken to be treated by the Folin-Wu method for protein-removal. After separating the precipitated protein by filtering, fractions were taken for analyses *a*, for copper-reducing substances, done by the Shafer, Hartman and Somogyi method; *b*, for chloride, done by the Whitehorn-Volhard method. The procedures followed for both methods are those given by Peters and Van Slyke (1932). Since the quantities of proteins in the hepatic outflow during these experiments were always very small, fractions from the samples for potassium determination were taken from the supernatant fluid immediately after centrifugation, and the protein neglected in the analyses. The method followed for the K determination was that of Hubbard's special modification of the Kramer-Tisdal method as utilized by Snyder and Johnson (1938). All the above methods were thoroughly mastered on controls and finally calibrated by duplicate analyses on solutions of known composition before the experiments were begun.

During the collection of the original samples at the hepatic outflow graphic records on a smoked drum, driven by synchronous motor, were always taken. The records included a time trace, signal from tipping-vessel at the outflow (giving thus rate of outflow), inflow pressure-level and the tracing of the calibrated plethysmograph (showing changes in the volume of the liver). The apparatus required for these graphic records is described in greater detail partly by Snyder and Johnson (1938) and partly by Snyder (1938). In this latter paper it was shown that the algebraic sum of total volume of hepatic outflow and total liver volume change, taking place during the collection of each sample, gives the corresponding total inflow volume from which finally the mean *minute volume inflow* to, as well as mean *minute volume outflow* from, the liver occurring during the collection of each sample can be calculated.

After the chemical analyses were completed the concentrations of the substances per unit volume for each sample were calculated, from which

one determines 1, the gain or loss in concentration per unit volume between inflow and outflow, and 2, the absolute amounts present in the outflow-samples and their corresponding inflow volumes. The differences between these inflow and outflow quantities, divided by the time required to collect the samples, gives in each case the mean gain or loss of the substance in the output per minute, as compared with the minute input to the liver. When the data are all tabulated one can easily see whether the gains and losses in concentrations between inflow and outflow parallel the gains and losses in minute volumes between inflow and outflow or not.

The above procedures were carried out both in control experiments, where all the conditions were kept as constant as possible, and in experiments where the drugs, acetyl-β-methylcholine chloride (AβMC) and epinephrin in the form of adrenalin-chloride (Adren.) were injected into the inflow.

An illustration of the smoked drum record of two such experiments will be found in the paper by Snyder (1938). The tabulated results of the chemical analyses of a number of the experiments as outlined above have been transferred to coördinate paper of which figures 1, 2 and 3 in this report are illustrations.

It was observed that the livers of fasting (hibernating) turtles, when placed in the oncometer tended steadily to gain in volume during perfusion. A number of experiments on such preparations were carried out during January, but their results will not be considered further in this place. The livers of well fed and active animals, however, when successfully transferred to the oncometer, maintained a rather steady volume for the duration of the experiment and as long as conditions were kept constant. It is the results of our experiments on these latter preparations that are here dealt with.

It was thought that probably the liver cells are more permeable to chloride than other ions in the perfusate under various conditions and that a knowledge of the concentrations of this ion would be an index as to the fluid transfers across cell membranes. In this series of experiments however the chloride was followed in only four experiments, the results of all of which appear among the figures.

The experiment of figure 1-A is a control during which the volume of the liver (naked dots, curve V) remained constant spontaneously. But, as the plots in crosses show (curve IV) the rate of inflow does vary somewhat (spontaneously) and hence the minute output varies accordingly in its gains and losses (from sample to sample) as the curves for K, sugar and chloride all indicate. However if only the change in outflow rate caused these differences, the curves of all three of those plotting minute-output gains and losses should follow the minute-volume outflow curve more closely than they do. It will be noted that the K and sugar curves follow

the minute volume curve rather closely but the chloride curve deviates at two points rather strikingly, taking a direction opposite to those of the sugar curves. Does this mean that, as sugar is taken up by the liver cells, chloride must be given up and *vice versa?*

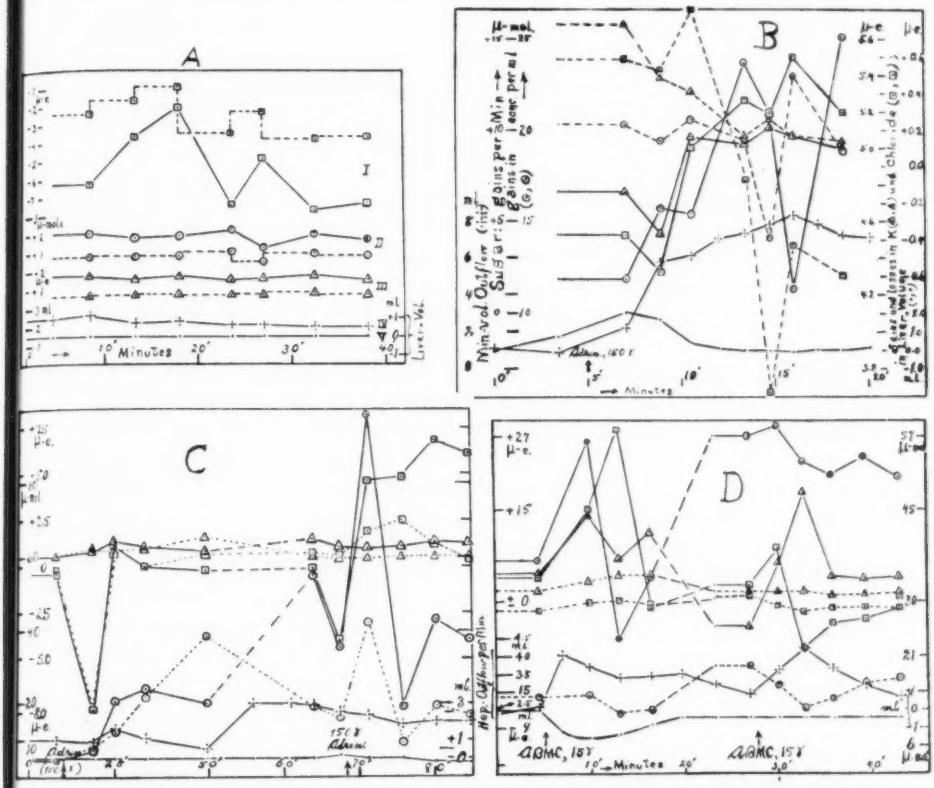

Fig. 1. Part A contains the data for the experiment of 4/4/38. This is a control experiment during which liver volume (curve V) remained spontaneously constant. In part C the experiment falls in two parts; the samples between them (nos. 6 and 7) were not analyzed. Likewise in part D, between the two parts, sample 13 was not analyzed. For additional explanatory notes see Addendum.

In the experiment, results of which are plotted in figure 1-B, Adren.-HCl was injected at the outset. The liver volume had already been increasing, but by the time the drug enters the liver sinuses (end of second sample) the volume begins to *decrease*, continues to decrease until the sixth sample is taken, and then remains rather stationary to the end. On the other hand the minute volume outflow begins to *increase* in response to the

drug and continues to do so up to the eighth sample. It will be noted that the excess of minute output over minute input of K, sugar and chloride increases in general with the increase of volume outflow whereas at the same time the *concentrations* of these substances per ml. in the output steadily falls below the concentrations in the input to about the seventh sample (curves in broken line). From this it appears that the increased outflow from the liver was due in part to water being given off by the liver cells which would explain the decrease in concentrations in the outflow as compared with the inflow. The excess output of sugar per minute is relatively much greater than that of chloride, when one considers their relative concentrations in the perfusing fluid (*ca* 1:6).

But as the results in another experiment (fig. 1-C) show Adren. may affect minute volume outflow only, and the liver cells apparently not at all. Here minute volume output and concentration curves follow each other for the most part rather closely.

When a minimal dose of AβMC is added to the inflow of the liver preparation, as shown in the experiment of 6/10/38 (fig. 1-D) the decrease of liver volume may be accompanied by a sudden increase in minute volume output. The graphs of the sugar, chloride and K show gains at this time both in concentration per milliliter and in amounts put out per minute. The losses following the gains appear to be all out of proportion to fall in rate of outflow. The sugar in response to the 2nd injection of AβMC, both in wt./ml. and wt./Min., shows a drop while the min.-vol.-output is again rising. The final drop in the hepatic output of sugar following the two AβMC injections, both in concentration and minute output is confirmatory of previous observations on the effect of the choline drug on hepatic outflow of sugar (Snyder and Johnson, 1938). The drop in concentration along with drop in minute output appears to point to absorption by the liver cells of both sugar and fluid, although conversion of sugar into lactic acid or other metabolites is not excluded here, as a possible cause of apparent fall in output of sugar. A variety of responses to AβMC in other liver preparations is shown in figure 2 and, following a previous injection of Adren., again in figure 3.

In table 1 are assembled what appear to be the maximum effects produced by the drugs in the various experiments. Here the changes produced are expressed in terms of per cent gain (+) or loss (−) over or below those prevailing just before the injections were made:

The response to epinephrin. In the lower half of the table are shown the maximum responses to Adren. It will be noted that the amount of copper reducing substances in the outflow generally is increased over that of the inflow (as was to be expected), not only when determined per minute volume but also per unit volume.

There is one experiment however for each of these two categories where

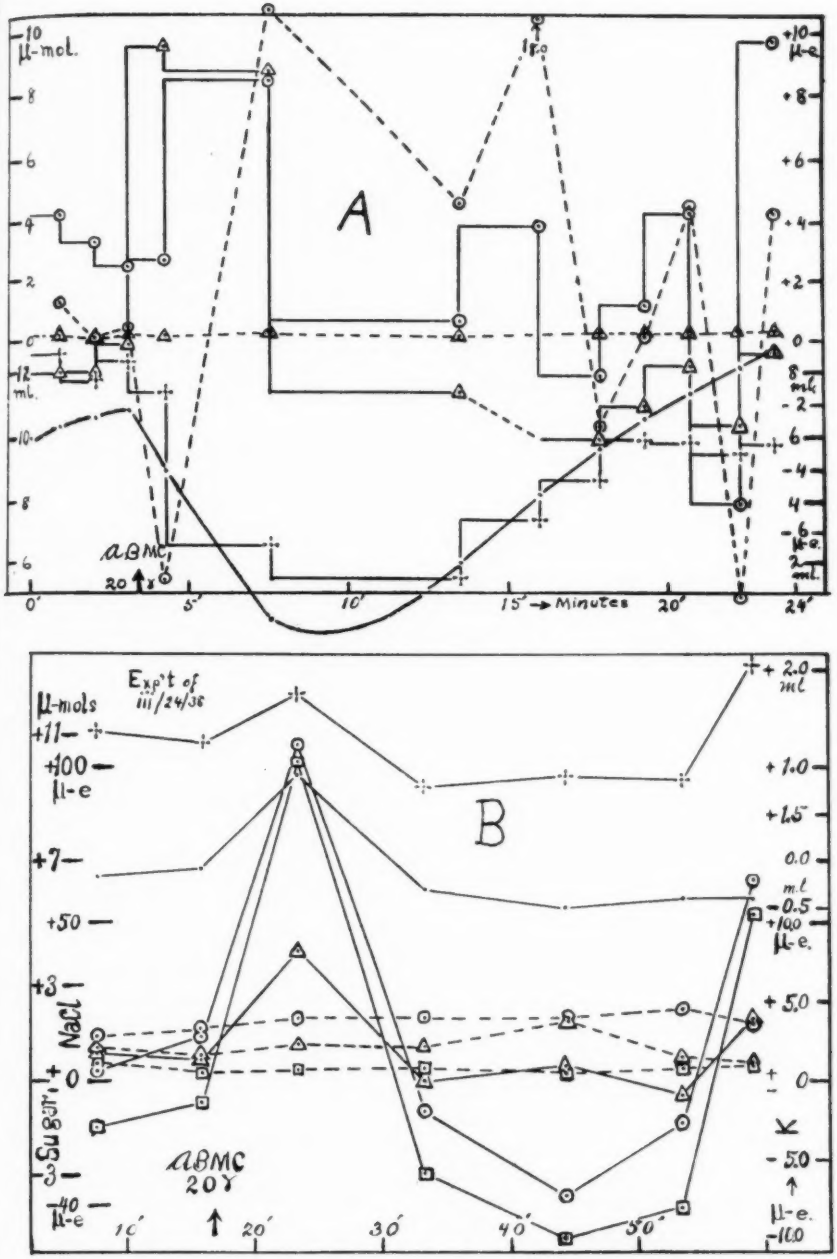

Fig. 2. Part A. The maximum decrease in liver volume here is 7.8 ml. and represents nearly 19 per cent of its original volume, 38 ml. The gain and loss of concentration per milliliter of the sugar undergoes tremendous oscillation, whereas that of the potassium remains practically constant. The fraction of sample 7, while being analyzed for K, was lost and does not appear in the K curves. See also Addendum.

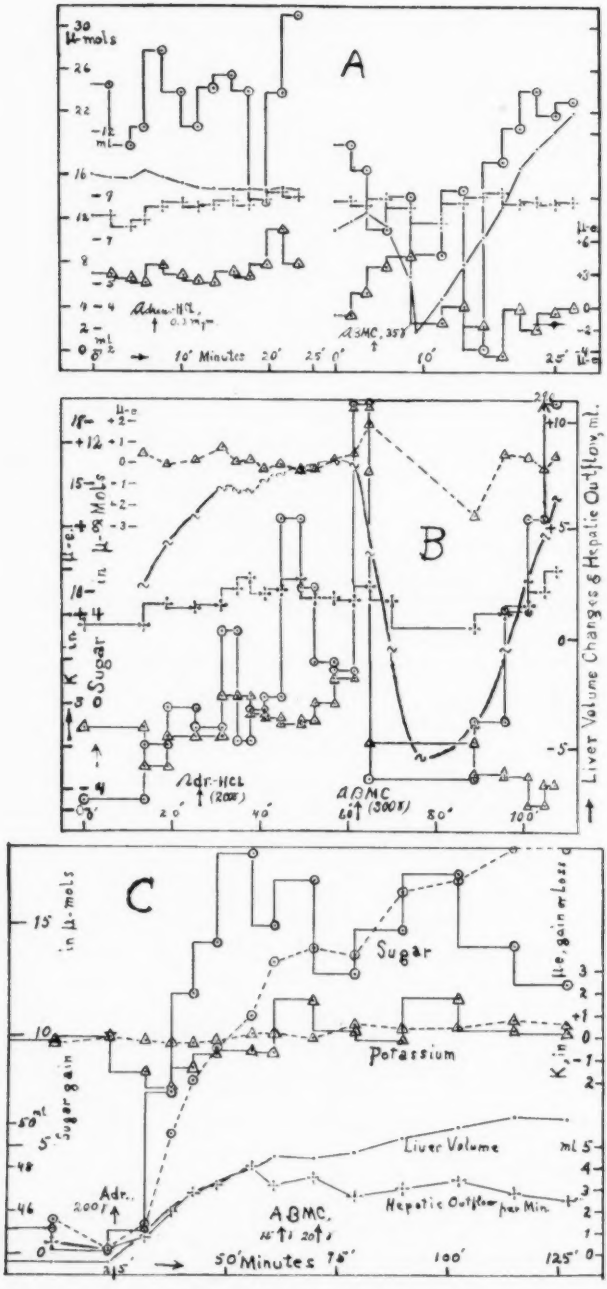

Fig. 3. In part A the outflow is plotted in absolute volumes per sample (which shows the chance range of the tipping vessel volume) and not in terms of minute volumes, and the volume of the liver at start is arbitrarily put at 10 ml.; the actual volume was *ca.* 49.5 ml. In part B the liver volume is plotted in circumflex symbols instead of naked dots. For additional notes see Addendum.

the output in sugar is less than the input. In the experiment of 6/3/38 it may be that the carbohydrate store already had been exhausted by the first treatment with Adren. In the experiment of 2/4/38 the value, −1.0, lies well within the limits of error and the deviation in the control experiment.

TABLE 1

DRUG USED	DATE OF EXPERIMENT	CHARACTER OF PERFUSING FLUID			DOSE OF DRUG	MAXIMUM EFFECT OF DRUG IN TERMS OF PER CENT GAIN OR LOSS (+, −) IN THE CHANGE OF OUTPUT OVER INPUT					
						Potassium		Dextrose		Chloride	
		pH	Dextrose	K		Per ml.	Per min.	Per ml.	Per min.	Per ml.	Per min.
			per cent	*per cent*	γ						
AβMC	6/10/38‡	7.3	0.133	0.016	(1) 15	+6.0	+197	−2.6	+53	+41	+600
					(2) 15*	−35.2	+1390	−6.0	−12	+41	−160
	3/24/38‡	7.55	0.143	0.020	(1) 20	+31.0	+437	+8.6	[+1240]	+1	+1040
					(2) 20	+1.7	−101	+7.7	−135	+31	−150
	2/25/38	7.55	0.156	0.022	30	−12.8	+300	−9.0	−220	†	†
	12/17/37	[7.5]	0.041	0.031	20	[+1800.0]	+330	[+245.0]	−17		
	12/ 4/37	7.55	0.065	0.030	33	−19.6	+1070	−18.5	+190		
	11/11/37	7.20	0.052	0.021	20	+2.8	+1420	−24.0	+192		
	11/ 5/37	7.2	0.056	0.022	20	+1.3	+470	−27.7	−73		
Mean..						+177 [−3.8]	+734	+11.4 [−14.6]	+112 [−12]	+28.5	+356
Adren	6/ 3/38‡	7.3	0.145	0.016	(1)150*	+21.5	+49	+40.0	+310	+0.1	+127
					(2)150	−0.2	+35	+28.0	−72	+1.7	+2170
	5/ 3/38‡	7.3	0.125	0.016	150	[+456]	−480	+22.2	+908	+31.0	−292
	3/ 3/38	7.55	0.162	0.016	300	+0.3	+46	+5.8	+316	+5.2	−26
	2/ 4/38	6.9	0.135	0.023	200	+1.0	+236	−1.0	+200		
	12/ 4/37		(Vide supra)		200	+4.3	−380	+40.0	+240		
	12/17/37		(Vide supra)		200	0.0	[−1340]	[+1180]	[+2230]		
	11/18/37		(Vide supra)		300	−4.1	+201	+16.8	+48		
Mean.						+60.0 [+3.2]	−183 [−16]	+167 [+21.7]	+523 [+279]	+8.8	+495
Control	4/14/38‡	7.45	0.117	0.021	0	±3.8	−8 +18	±7.8	+19 −52	±2.8	−48 +21

* In the cases marked thus the liver volume was kept constant by clamping off the outlets from the extrahepatic space of the oncometer.

† A blank space indicates no analysis was made.

‡ In the experiments marked with ‡, the analyses were all made in duplicate.

It will be noted that, if the largest value in each of these two columns is excluded (the bracketed figures), then the mean values for the remaining cases are still positive and of magnitudes much greater than occur in the control.

The analyses for K show a variety of effects. In three experiments the values for output in unit time are large and negative in sign. Those with positive sign are more numerous but smaller. The mean of all how-

ever is still negative. The mean value of the concentrations per unit-volume however is positive. But if the largest value in each rubric is discarded the mean values of the remaining cases, although keeping their sign characters, still are so small as to fall within the limits of changes that may have occurred had no drug been given. (Compare the bracketed mean value of K with the K-values in the control experiment.) We must conclude therefore that so far as these experiments go epinephrin does not affect the K-output from the turtle's liver in any outstanding way. There is only a tendency, when the effect is at its maximum, for the outflow per minute volume to hold less K than the corresponding minute-volume-inflow. These findings agree with those found by Snyder and Johnson (1938) in experiments where account was taken only of rate of outflow and not also of volume changes (and inflow volumes) of the livers.

The response to acetyl-β-methylcholine. The data in the upper part of the table reveal less concordant results in the maximum effects of AβMC upon the liver responses. While six out of the ten experiments show a maximum effect of sugar-loss in the minute output (compared with minute input), among the other four which show a gain in the output one of them has a positive value so large that the mean of all ten has a large positive value. Discarding this very large positive datum, the mean of the remaining nine now becomes negative; but of such small magnitude that it falls within the limits of variation found in the control experiment. On the other hand when the gain or loss in the *output per unit volume* is compared with *input per unit volume*, one finds negative values in seven out of the ten experiments. Discarding again the most divergent datum (also a positive) the mean of the remaining nine is of negative sign, but this time somewhat outside the limits found in the control. One may conclude then that the experiments show a tendency for the sugar in the hepatic outflow to be reduced below that of the inflow under the influence of AβMC, but not always by any means.

The effect of the choline drug on the output of potassium from the liver is much more constant. Here the K-excess in the minute-volume output over the minute-volume input is positive in all cases except in one of the trials on one of the livers. This gain when expressed as percentage is consistently large.

When only concentrations in contents of unit volume are compared we find only one case where the figure is very large and positive. Discarding this large datum again the mean value of the others (all of which are relatively small and of various sign) we have a small value of negative sign falling within the extreme deviation observed in the control. The pronounced increase in potassium output per minute volume, as here calculated on the basis of liver-volume changes, is a result just the opposite of that found by Snyder and Johnson (1938) in experiments where no account

was taken of liver-volume changes. It is a remarkable demonstration of the importance of observing organ-volume changes when one wishes to know the real output per unit of time into the general circulation and thus, more intimately, the nature of the effect of organs on the body economy.

The outstanding results of this investigation may be described as follows:

1. The volume changes of the liver, combined with the changes in the rate of flow through the organ, have marked effects upon the quantities of substances put out or held back from the fluids perfusing the organ during relatively short periods of time.

2. When external conditions are kept constant, as is to be expected, the difference between uptake and output shows least deviation (see fig. 1-A).

3. Occasionally however marked changes may occur, apparently quite spontaneously, causing rhythmical changes in liver-volume which become less pronounced the deeper into the liver one inserts the outflow cannula.

4. In response to AβMC and Adren., given in small doses by injection at the inflow cannula, the volume changes, as well as changes in the rate of outflow, may become very marked. (See also Snyder, 1938.) The differences between minute-volume outflow and minute-volume inflow at such times becomes very large. While both drugs usually cause decrease in liver-volume, AβMC at the same time usually causes a decrease in minute-volume outflow, and Adren. usually causes an increase in minute-volume outflow. The gain and loss therefore in the absolute amounts of substances, poured out with the hepatic outflow in unit-time, may undergo likewise very large alterations, and this even at a time when their concentrations undergo little or no change in passing through the organ. (See fig. 2-B and fig. 1-D, especially for K and chloride, and compare also with data in table 1.)

5. There is a tendency for the response to be oscillatory in character. If the immediate effect of a drug is a decreased output, a wave of increased output follows to be succeeded by another wave of decrease, and *vice versa*. These oscillatory responses are not always related with volume changes of the liver or changes in rate of hepatic outflow; they may even appear in control experiments, although with less intensity, as is shown in figure 1-A. In all such cases the cause of the changes is probably due to transfers in and out of the tissue cells. For,

6. In the experiment, figure 1-B, in response to Adren. it will be noted that the gain in sugar in minute output over the input increases markedly as does also the chloride. This is due to increased rate of perfusion in spite of the head of inflow pressure remaining constant. But the principal change in the concentration of the sugar is one first of decrease; at the same time the concentration of the chloride at the outflow suffers also an enormous decrease and that of the K a marked decrease.

Also in the experiment of 12/17/37 (fig. 3-C), where the gain and loss in

concentration per milliliter of sugar and K are plotted along with the gain and loss per minute outflow, we have another illustration of the combined vascular and tissue-cell responses to the drugs. The concentration per milliliter of K drops a very little under Adren. and rises a little more under AβMC but both these effects on the output are reinforced by the effects on the vascular responses. The sugar both in unit-volume and in unit-time of the output are both greatly increased by Adren. which is not reduced by AβMC.

It would be difficult to explain these changes in concentration other than by assuming that epinephrin either facilitates transfer of water out of the liver cells (Lesser) or causes fluids in the tissue spaces, poor in the substances analyzed, to be transferred to the hepatic outflow. This would account also for part of the increased outflow over the inflow during the contraction of the liver-volume, as seen in figure 1-B. The steady gain in liver volume in figure 3-C, as well as the continued rise in sugar output must be due to the slow rate of perfusion, the probable partial asphyxia thereby produced causing general vascular dilatation, as well as glycogenolysis.

7. The reverse state of affairs takes place in the experiment plotted in figure 2-A, where the liver is responding to AβMC. Here the minute output of sugar and K drops below the minute input very markedly while the concentration of sugar in the output, after a brief fall, rises quite as markedly only to fall again later in the recovery, during which time the concentration of K in output and input suffers no change at all.

8. That the tissue cells probably do not always take part in the response appears in the record of the experiment figure 2-B. Here the first effect of AβMC is to increase the rate of outflow and decrease liver volume. With this vascular response the minute output of sugar, chloride and K is greatly increased over the input; and then, with the increase in liver volume and decrease of rate of outflow, there is synchronously a return of the minute output of these substances. But notwithstanding all these apparent vascular changes the concentrations of the three substances per unit-volume outflow remains nearly the same as that of the inflow. In short we have here an experiment where apparently the choline drug affects the walls of the vascular bed but the cells of the liver not at all.

SUMMARY

Analyses of the inflow and outflow to a liver preparation surviving in an oncometer have been made for K, sugar and, in a few experiments, also for chloride.

The samples analyzed were collected from the hepatic outflow at frequent, successive and short-lasting intervals of time while the organ was

responding to injections of acetyl-β-methylcholine chloride or adrenalin chloride into its inflow cannula.

It is shown that, when the changes in volume of the organ as well as changes in rate of outflow and inflow are taken into the calculations, then the amounts of uptake and output of substances contained in the perfusate may be quite different from those indicated by concentrations alone at the sites of inflow and outflow. The differences in content per unit of time under certain conditions may be all out of proportion to the differences in content per unit of volume, and the values for the former may change greatly while those for the latter change not at all and *vice versa*.

It is pointed out that the method obviates resort to the procedure employed in chronic cases, namely, the taking of sections out of the organ under investigation. The method further makes possible the experimental differentiation between the vascular and glandular effects of various agents upon the liver.

The experiments here reported demonstrate this differentiating capacity of the method, especially when autonomomimetic agents are injected into the portal vein of the liver.

The method promises further to be useful both in *a*, the study of the rôle played by an organ in producing transient alterations in the composition of the general circulation (minute-volume output here is of the greatest importance); and in *b*, the study of the rôle played by the varying contents of the general circulation, or of affluent nutrient fluids, upon the organ itself in both its vascular and glandular responses.

REFERENCES

PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. II. Methods. Baltimore, Md., 1932.

SNYDER, C. D. This Journal **124:** 647, 1938.

SNYDER, C. D. AND R. E. JOHNSON. Bull. Johns Hopkins Hosp. **62:** 110, 1938.

ADDENDUM

Explanatory note to figures 1, 2 and 3. These figures contain the final results of a number of experiments perfusing turtles' livers surviving in an oncometer. The abscissae in all the graphs represent time in minutes, reading from left to right. Along the ordinates are plotted the quantities derived from drum records and from chemical analyses of the perfusates. The symbols used in the curves fall along the time axis at the moments when the samples of outflow of constant volumes were collected and are uniform throughout:

1. The *naked dots* connected in full line = changes in liver volume in milliliters.

2. *Crosses* connected in full line = rate of hepatic outflow in terms of milliliters per minute. All other curves contain the data calculated from the chemical analyses (for sugar, potassium and chloride) in two categories.

(a) Those connected in full line state the *gains or losses* in the *minute volume* hepatic vein outflow as compared with the portal vein inflow per minute volume.

(b) Those connected in broken line state the *gains and losses* in the *concentrations* per milliliter of the outflow as compared with milliliters of the inflow to the liver.

In general:

3. *Circles* = sugar, in micro-mols.

4. *Triangles* = potassium, in micro-equivalents.

5. *Squares* = chloride, in micro-equivalents. For the exceptions consult below under heading "Special comment . . . " The upright *arrows* along the abscissae indicate the times when injections were made into the portal v. can. by Luer syringe of Adren. or of the choline drug, AβMC; the absolute weights being usually stated in the charts (in gammas). The analyses being made of samples collected in constant volume of outflow are therefore the mean values for the time required for collecting the volume. The lines connecting the data should therefore be rectangles (for the sake of accuracy) rather than diagonals. But to simplify the work now and then diagonal connecting lines are used in plotting the curves. For additional information, absolute volumes of liver preparations, character of perfusing solutions, etc., consult Snyder (1938, table 3) and text and table 1 of this paper.

POTENTIAL CHANGES IN AN INJURED REGION OF CARDIAC MUSCLE[1]

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Since an injured region of ventricular muscle does not contract when the rest of the muscle becomes active, it has been generally assumed that such an area is, with the exception of the constant negative injury potential present during rest, electrically inactive. This assumption has led to the widespread use of bipolar leads from injured and uninjured surfaces with the idea that the potential changes recorded during activity are to be referred solely to the uninjured region. The curves were supposed to give a true record of the monophasic negative change assumed to occur in active tissue under the almost universally accepted negativity theory of tissue potentials. A recent comprehensive review of the whole subject and an analysis of the immense amount of work that has been done on this assumption is given by Schütz (1).

The first direct attempt to determine whether or not an injured region changes in potential during activity of the surrounding muscle, was made by the use of a unipolar lead by Wilson, Macleod, Johnston and Hill in 1933 (2). They showed that the resting negative potential of an injured region on a tortoise ventricle disappeared during activity. Our own work has confirmed this result and in addition shows that the injured region becomes electrically positive with respect to quiescent muscle when the surrounding muscle enters into activity.

METHODS. The experiments were carried out on tortoise hearts in situ, or isolated at the center of a large circular conducting field (3), and on the dog's ventricle in situ. Records of injury resting (irp) and injury activity (iap) potentials were made with one or two string galvanometers, or with one or two cathode ray oscilloscopes, recording simultaneously. In both cases direct coupled amplifiers were employed. In recording the potential changes in the injured region, unipolar leads were used in most cases. One electrode led from the heart, the other from a distant point in the body, or from the margin of the circular field in the case of the isolated tortoise heart. The second galvanometer was used for recording either an electro-

[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation.

gram, electrocardiogram, or the current changes induced in a photoelectric cell by the shadow of the moving heart.

Local areas of injury were produced in the tortoise ventricle by burning with a hot iron, by crushing with forceps, or by the use of the suction electrode described by H. Wiggers (3). The suction electrode alone was used in the dog and gave consistently satisfactory results.

Injury potentials of rest (irp) and activity (iap). Before describing the potential changes occurring in an injured region of cardiac muscle during activity of the surrounding uninjured region, we wish to make clear that in all cases the reference level of potential is the potential of the uninjured resting muscle of the experimental preparation employed. With this in mind, it is easy to show that an injured portion of heart muscle, far from being a region which, according to the prevalent conception maintains a constant potential and may thus serve as an indifferent lead in recording action potentials from an uninjured region, develops a potential change of relatively large amplitude when the surrounding muscle goes into activity. This change in potential is in the positive direction and its magnitude is sufficient not only to obliterate the negative potential of the injured region present when the heart is at rest, but to cause the region to become strongly positive with respect to the reference level of potential. Records demonstrating this potential change in the injured region are shown in figure 1. A resting negative potential (irp) of -10.3 mvs. is not only obliterated but replaced by a positive potential of $+8.2$ mvs.

According to the prevalent conception of constant potential in an injured region unaffected by activity of surrounding muscle, no potential change should show in a record obtained from leads from two separate regions, both injured, except relatively small changes of the normal action potential type arising from active muscle lying between the two electrodes. The same result would also be obtained if the potential changes were exactly the same in magnitude and in time relations under each electrode. Since electrical activity however does occur in each injured region, and since the magnitude of change in two different regions of injury is in general different, the curves always show potential change of the injury type. The direction of the curve will depend upon the relative magnitudes of the iap developing in the two injured regions.

The magnitudes of the resting and activity injury potentials are quite variable, depending in part at least on the type of injury, as shown in table 1.

The figures under irp and iap are in millivolts with reference to the potential of the uninjured resting muscle, the former in all cases negative, the latter in all cases positive. The total potential change during activity is really the sum of two parts, that which obliterates the resting potential which is negative with respect to resting uninjured muscle, and a further

potential change which makes the injured region positive with respect to the same reference. Only the latter is recorded in the table. The largest

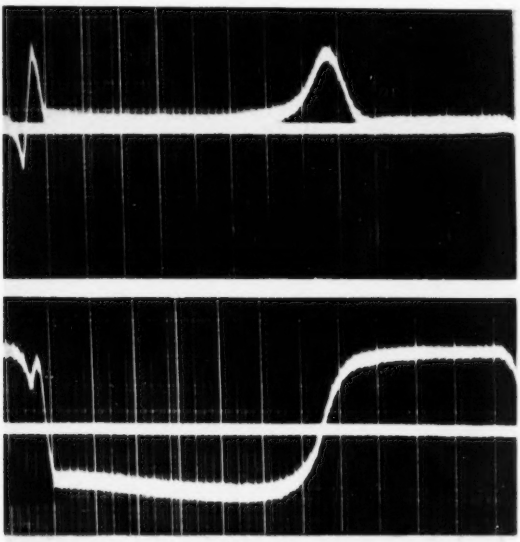


Fig. 1

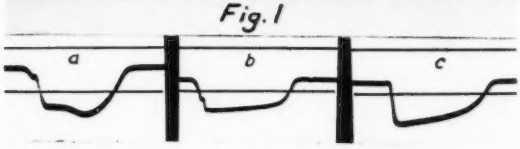


Fig. 2

Fig. 1. Potential time curves from a tortoise heart in situ, one electrode on the mid anterior ventricle, the other on the muscles of the left hind leg. The upper curve is recorded before, the lower curve after the region under the electrode was burned with a hot iron. The horizontal line in each case represents the position of the galvanometer thread when the cardiac electrode is on resting uninjured ventricular muscle. Any deviations above this line represent negativity, and any deviations below positivity, with respect to this reference. For further description, see text. Time intervals in 0.04 sec.

Fig. 2. Records from three types of injuries produced in the same tortoise ventricle. The curves are records of injury resting and activity potentials, recorded from one lead from the injured region, the other from an electrode on the muscle of the hind leg. The horizontal line represents the position of the oscilloscope spot when the cardiac electrode is at the potential of uninjured resting muscle. Above this line represents relative negativity, below relative positivity of the cardiac electrode. For further description, see text.

potential changes are in general obtained when the injury is produced by the suction electrode, particularly in the dog's heart.

As is well known, the negative potential of injury declines with time, at first rather rapidly, later somewhat more slowly. The same is true of the positive phase of the activity potential, although in most cases the decline is slower and the ratio $\frac{\text{positive phase of iap}}{\text{irp}}$ increases. Finally, the injured region may show a potential change resembling the normal action potential, but differing in certain respects from the action potential obtained from the same region before it was injured, particularly in the absence of an isopotential period and the invariable presence of a large positive T wave. This form is quite independent of the form of the action potential obtained before injury.

The form of the iap is somewhat variable, depending to a large degree at least upon the nature of the injury. In figure 2 curve *a* is a record of potentials from a burned area, curve *b* the same from a crushed area, and curve *c* the same from an area in which injury was produced by suction

TABLE 1

Injury resting (irp) and injury activity (iap) potentials

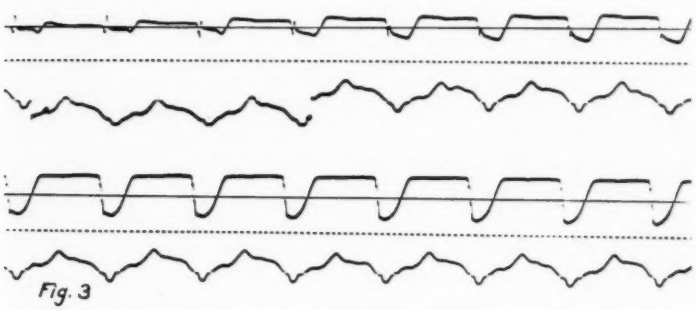
ANIMAL	NUMBER OF EXPERIMENTS	TYPE OF INJURY	IRP			POSITIVE PHASE OF IAP			IRP > +IAP	IRP = +IAP
			Average	Maximum	Minimum	Average	Maximum	Minimum		
Tortoise	23	Burn	12.4	21.1	4.6	10.2	20.0	2.0	14	0
Tortoise	10	Crush	14.4	22.4	9.5	13.6	16.8	10.0	5	1
Tortoise	13	Suction	15.1	30.0	5.0	30.3	46.0	17.0	1	3
Dog	23	Suction	26.1	48.0	12.0	31.1	48.0	20.0	5	6

with a Wiggers electrode. All curves were obtained from the same heart. The total potential changes (irp to maximum of iap) were 42.2, 44.0 and 80.0 millivolts respectively.

The development of the resting and contraction potentials can be readily followed by taking a continuous record before and during the injury produced by a suction electrode. Figure 3 is an example. The suction was started during the first cycle shown on the record. In the normal cycle, a diphasic QRS is followed by a diphasic T complex. In the next cycle the development of negative injury potential, following the T wave, is already apparent, reaching a magnitude of -15.0 mvs. early in diastole. In the third cycle, the irp has increased to -22.6 mvs., and in the fourth cycle to -27.0 mvs. In the fourth cycle the positive phase of the iap is definitely present, reaching a maximum of $+20.0$ mvs. Both phases continue to increase, and the action current component becomes less evident. Toward the end of the lower record, the irp has increased to -48.0 mvs., the positive phase of the iap to $+48.0$ mvs.

In similar experiments, both on the tortoise and dog ventricle, a change in the potential curve within the first cycle following the application of the

suction has always been present. Along with the growth of the irp and iap, there is a complete or nearly complete disappearance of the action potential recorded from the region before injury (fig. 4).



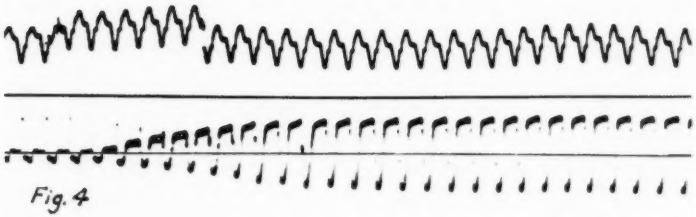


Fig. 3. Record of the development of injury resting, and contraction potentials in the exposed dog's heart produced by a suction electrode applied to a spot on the right ventricle. The upper record shows the initial stages, the lower, later stages; 12 cycles intervening between the two records. The horizontal solid line in the upper curves of each record represent the position of the oscilloscope spot when the cardiac electrode is at the potential of resting uninjured ventricular muscle. Deflections above represent relative negativity, deflections below, relative positivity, of the cardiac electrode. The distal electrode was in the muscle of a hind leg. The middle broken line in each case records time in 0.04 sec. intervals. The lower curve of each is a mechanogram recorded by the shadow cast by the right ventricle on a photoelectric cell, upstroke indicating systole. The onset and duration of suction is indicated by the two breaks in the mechanogram in the upper record.

Fig. 4. Record of development of injury potential in the dog's ventricle as a result of application of the suction electrode, made at a slower speed of recording than in figure 3. The upper curve is the mechanogram of the ventricle recorded by means of a photoelectric cell. The horizontal line in the lower curve has the same significance as described in the preceding figure. The early decline and nearly complete inclusion of the normal QRS and the symmetrical growth of the injury resting and contraction potentials are well shown. The injury resting potential shows an increase during each diastolic period. Both injury potentials are fully developed by the end of the record.

The potential changes in an injured region are confined to the newly developed irp and iap, and the region no longer produces the action potentials characteristic of normal muscle. This may be shown by the fact that when an electrode of the differential type makes contact within an area of injury, the potentials recorded are less than 2 per cent of those obtained from the same region before injury. This small residue is to be ascribed to neighboring active regions and represents the "extrinsic" potentials present when an electrode of this type is employed on uninjured muscle. The monophasic iap is not recorded under these conditions because both electrode contacts are within the area of injury.

Comparison of unipolar and bipolar leads. In the experiments described above, unipolar leads were employed in all cases. One electrode was applied to the heart, the other to a distant point in the animal, or to the margin of the field in the case of the isolated tortoise heart. It has been shown in the former case that the contribution of the distant electrode to the potential curve is very small (5), and in the latter case negligible (3). The potentials recorded are in larger part derived from the immediate region of contact of the cardiac electrode, but also in part from neighboring regions. It is the latter which contribute the action potential components, usually designated as extrinsic parts of the otherwise smooth monophasic curve obtained from an injured region. In the case of bipolar leads, where both electrodes are applied to the heart, one to an injured, the other to an uninjured region, the curve obtained is a composite of injury potentials arising from the injured area and action potentials arising from the uninjured area beneath the other contact and from other normal heart muscle between the two electrodes. Because of the rapid rise, the magnitude and long duration of the potential change (iap) in the injured region, one may however obtain curves which show little or even no apparent trace of the action potential derived from the other contact and the intervening uninjured muscle. This is particularly true if the speed of recording is slow and small irregularities of the curve are smoothed, and when the suction electrode, which produces the largest iap obtained in any form of injury, is employed. The action potentials of the normal uninjured regions may then be completely obscured by the large monophasic iap of the injured region.

In the great mass of work on injury potentials, bipolar leads have been employed, on the mistaken assumption that only the uninjured region contributes to the potential curve. That this assumption is incorrect and the contrary true, namely, that the curve so obtained is almost entirely due to potential changes occurring in the injured region, is readily shown by a comparison of curves obtained from bipolar and unipolar leads.

Consider two electrodes applied to the tortoise heart, one near the base (B), the other near the apex (A) and a third electrode applied to a posterior

limb (F). The circuits are so arranged that potential-time curves can be recorded between the ventricular base and the leg (BF), between the apex and the leg (AF) and between the two electrodes on the heart (BA). A record of ventricular contraction is recorded simultaneously with each of these curves for time reference. Further, the connections are such that an upstroke on the record occurs when B is negative with respect to F,

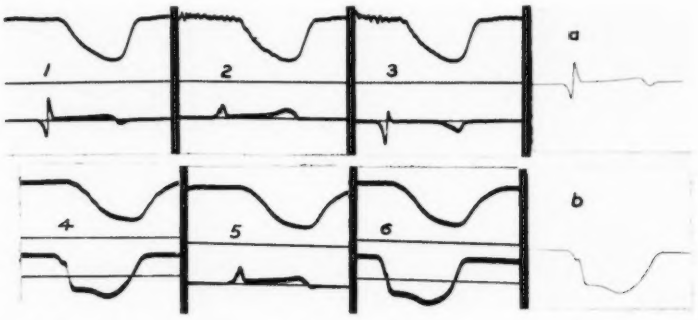


Fig. 5. Records obtained from a tortoise ventricle to show the relation of unipolar and bipolar leads. In each of the six records the upper curve records the mechanical activity of the ventricle, downstroke representing systole. The lower curves in each case represent the potential changes from unipolar or bipolar leads from the heart. Each dot in the midline of the records represents time in 0.04 sec. intervals. For further description, see text.

when A is negative with respect to F and when A is negative with respect to B. From Kirchhoff's e.m.f. law

$$\begin{aligned} AF + FB + BA &= 0 \\ \text{or } -FA + FB + BA &= 0 \\ FB + BA &= FA \end{aligned}$$

The addition of the curves obtained from the leg-base and the base-apex lead should give the curve obtained from the apex-leg lead, if the same amplification is employed in each and simultaneous points are added.

Records from an experiment of this type before and after injuring the surface under the apical electrode are given in figure 5. Records labeled *1*, *2* and *3* were made from a tortoise heart before injury. Records *1* and *2* are from unipolar leads from the ventricular apex-leg (AF) and the ventricular base-leg (BF) respectively. Record *3* is from the bipolar lead between base and apex (BA). The three lower records were taken in the same way immediately after the region under the apical electrode was injured by burning. The two curves, drawn to the right of the records, and labeled *a* and *b*, represent the summations indicated above. The additions were made by optical enlargement of the curves and superposi-

tion with reference to the base lines and to the reference mechanograms. Within the errors of measurement, the summation curves are identical with the apex-leg curves. Thus *a* corresponds to *1*, and *b* to *4*. An experiment of this type shows clearly the composite factors in bipolar leads, the injury potential component from the injured region and the action potential component from the uninjured regions.

The curves recorded in figure 5 also illustrate the relatively small effect that a rather large action potential component may have on the injury potential curve. There is little obvious action potential component in the unipolar lead from the injured region (record *4*), despite the fact that this curve is formed by adding the bipolar lead from injured-uninjured surfaces (record *6*), which itself has little action component, to the curves formed from the uninjured base-leg lead (record *5*), which shows QRS and T complexes of considerable magnitude.

The origin of injury resting and activity potentials. The injury potential of rest, negative with respect to uninjured quiescent regions, has usually been explained as a result of the exposure of the negatively charged interior surface of the polarized membrane. Craib (6) has shown that a skeletal muscle injured at one end and immersed in a conducting bath, is relatively negative at the injured, relatively positive at the uninjured end, with reference to an indifferent point in the field, and the potential distribution in the field around the muscle accords with that of a dipole at the center of the field. Wilson, Macleod and Barker (7) have come to essentially the same conclusion in a theoretical consideration of the subject, based on the hypothesis of the polarized membrane. The theoretical interpretation at which they arrive would appear adequate to account for the presence of the negative resting potential and its disappearance during activity when surrounding normal muscle becomes depolarized. It does not appear adequate, however, to account for the development of a potential positive with reference to quiescent uninjured muscle in the injured region during activity of the surrounding muscle. The process appears more complicated than has previously been thought, and to warrant further experimental investigation. We have carried out the following studies. First, if an isolated quiescent tortoise ventricle is placed at the center of a large circular field and the potential field around it explored by methods previously described (3), the field is practically isopotential both before and after an injured area is produced, provided *a*, the injured region is not immersed, or *b*, if it is immersed, that the plane of the injury corresponds to that of the field. If the preparation is immersed, and the injury produced in a region such that it is not parallel to the surface of the field, a potential distribution is obtained which corresponds to that of a dipole. In a second series of experiments, carried out on the quiescent tortoise ventricle, either

in situ or isolated in the circular field, we have explored the potential distribution on the ventricular surface, including an area of injury. It was found that the negative potential of rest present within and at the margins of the injured region, is sharply circumscribed to the injured region, and although its magnitude may be considerable there is no appreciable spread to surrounding normal muscle. If the potential is measured in such a preparation at successive points, in the neighborhood and across the injured area, no change in potential occurs until a point is reached at or in the immediate neighborhood of the injury. The potential then becomes rather sharply negative and remains approximately constant across the injury, to return to isopotentiality with uninjured resting muscle when the margin of the injury is passed. There is at no point a region more positive than quiescent uninjured muscle. By repeating this experiment in hearts which were not quiescent but active, the distribution of the positive potential of activity (iap) was determined from potential time curves obtained from successive points as above. The distribution of the positive potential is essentially the same as that of the irp, it is confined to the region of injury and its immediate neighborhood, and at no place is there found a relatively negative potential during the stage of ventricular activity.

The experiments thus show that the irp is characterized by a negative potential state and the iap by a positive potential state, localized to the region of injury and its immediate neighborhood, both states being attained rapidly and both unassociated with any region in which a potential of opposite sign appears.

Having determined the characteristics of the potential distribution the next step in a problem of this sort is to attempt to determine what configuration of charges will give the potential distribution observed. If the potential distribution is known in accurate detail, and if a charge configuration that will give this particular potential distribution can be found, the problem is uniquely solved. This particular charge configuration is the one and only one that will give the observed potential distribution. It is found in the present case that the assumption of a charge configuration consisting of two concentric rings, the outer one containing positive, the inner one an equal number of negative charges, will give a distribution of potential such as we have observed experimentally. The assumption that the total charge, the sum of all the positive and negative charges, must be zero, is conditioned by the fact that the potential outside the configuration (outside the margins affected by the injury) is not influenced by the disposition of charges within the injured region or its immediate neighborhood. The mathematical formulation of the problem applying to the charge configuration indicated above, consists first in obtaining expressions repre-

senting the normal component of the electrical intensity (E_r) over different parts of the configuration by the application of Gauss' theorem, and deriving equations representing the potential by the use of the integral $V = \int_{\infty}^{r} E_r dr$; where r is the distance from the center of the concentric rings. The equations obtained are negative for all values of r less than the distance from the center to the outer margin of the outer ring. For all values of r greater than this, $V = 0$. Passing toward the center from the outside margin of the outside ring the negative potential increases as r becomes smaller over both rings of charges. Within the inside ring, the potential is constant and of maximum (negative) value.

The analysis of the assumed configuration of charges shows that this configuration will manifest the same potential distribution as we have found by direct measurement across an injured region. If our measurements are sufficiently accurate and taken from points sufficiently close together to assure us that our conclusion as to the potential distribution across the injured region is correct in detail, the charge configuration described must be the one that is actually present. The number of separate spots from which the potential can be measured in the biological preparation is of course limited, and it is quite possible that the actual charge configuration in the injury differs in certain details from that given. The charge density may be not uniform as assumed in the formulation but variable. The essential characteristics will, however, be maintained.

With the conception of the electrical pattern responsible for the resting injury potential established, it is to be noted that the reversal of polarity that occurs when the surrounding muscle becomes active, the occurrence of the positive phase in the iap can only occur by a reversal of charge distribution, the outer portion of the injured region now containing negative, the inner portion positive charges. On the assumption (as in the membrane theory) that the electrical charges responsible for tissue potentials are entirely carried by ions, the polarity reversal would of necessity be ascribed to ionic interchanges, taking place at the margin or within the injured area. When the ventricle becomes active there is a rapid reversal of ionic distribution abolishing the irp and giving rise to the iap. At or toward the end of ventricular activity the distribution is again reversed, the iap disappearing and the irp being again established.

On the other hand it may be that there are sources of electricity present other than those arising from ionic configurations and that a new charge configuration does not necessarily imply an equivalent interchange of ions.

SUMMARY AND CONCLUSIONS

1. The potential changes in an injured region of ventricular muscle of the tortoise and dog heart are studied during rest and activity of the surrounding normal muscle. The reference level of potential is taken as

the potential of uninjured resting ventricular muscle. With this reference it is shown that the negative potential of rest is replaced by a positive potential during activity, the injured region becoming positive to a degree which may be as great or greater than it was negative during the preceding stage of inactivity of the surrounding ventricular muscle.

2. The near or complete absence of action potentials in an injured region, as shown by the use of a differential electrode, and the magnitude and duration of the positive potential occurring in such a region during ventricular activity, point to the presence of some new source of potential in an injured region which is not present in uninjured heart muscle.

3. The largest potential changes and the most consistent form of potential time curves from an injured region were obtained when the injury was produced by the suction electrode devised by H. Wiggers. With such an electrode the development of the injury potentials can be followed in successive cycles. The fully developed potentials are established within a short period, somewhat more rapidly in the tortoise than in the dog heart.

4. The injury potentials recorded from unipolar leads, with one electrode on the injured surface, the other at a distant point, resemble closely those obtained from two contacts on the heart, one on an injured, the other on an uninjured surface.

5. In bipolar leads from injured to uninjured surfaces on the heart, the large and prolonged positive potential developing in the injured region when the heart becomes active, nearly or completely obscures the normal action potential developing in the uninjured portions of the muscle. The large monophasic curve, which has been assumed to result from the occurrence of a negative potential change at the uninjured surface, in reality results from the positive potential change developing on the injured surface.

6. The potential distribution across an injured region on the quiescent tortoise ventricle shows no region which is positive with respect to uninjured quiescent muscle. The potential is uniform up to the margin of the injured area, becomes rather abruptly negative and remains negative and approximately constant until the opposite marginal tissue is reached, returning to its original value beyond the injury. This distribution of potential accords with that of two concentric rings, the outside ring containing positive, the inside ring negative charges. This orientation of charges is reversed when the surrounding muscle becomes active.

REFERENCES

(1) Schütz, E. Ergebn. d. Physiol. **38**: 493, 1936.

(2) Wilson, F. N., A. G. Macleod, F. D. Johnston and I. G. W. Hill. Proc. Soc. Exper. Biol. and Med. **30**: 797, 1933.

(3) Eyster, J. A. E., F. Maresh and M. R. Krasno. This Journal **110**: 422, 1934.

(4) WIGGERS, H. C. This Journal **118**: 333, 1937.
(5) WILSON, F. N., S. W. WISHART AND G. R. HERMAN. Proc. Soc. Exper. Biol. and Med. **23**: 276, 1925–26.
(6) CRAIB, W. H. J. Physiol. **66**: 49, 1928.
(7) WILSON, F. N., A. G. MACLEOD AND P. S. BARKER. The distribution of the currents of action and of injury displayed by heart muscle and other excitable tissue. Univ. of Mich. Press, Ann Arbor, 1933. Also J. Gen. Physiol. **16**: 423, 1933.

THE INSENSITIVITY OF DIVING ANIMALS TO CO_2[1]

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The breathing of mammals is so sensitive to CO_2 that the addition of 0.2 volume per cent to the alveolar air will double the rate of pulmonary ventilation (Haldane and Priestley, 1905). Internal respiratory adjustments which involve the heart and circulation are also quickly set in motion by CO_2, and sensitivity to CO_2 is regarded as the most conspicuous single agent in the control of mammalian respiration.

In the following experiments it is shown that in beaver and muskrat the breathing, the action of the heart, and certain reactions of the peripheral circulation are relatively insensitive to CO_2. In spite of their insensitivity to CO_2, respiratory adjustments of diving animals to sudden asphyxia are prompt and effective. In diving animals some of the characteristic and generally important physiological responses of mammals to acute asphyxia cannot be attributed to stimulation by CO_2, and several details of the mechanisms which are involved in meeting sudden asphyxia are clearly revealed by comparison of divers with land animals.

METHODS. The animals, beaver, muskrats and cats, were prepared under chloralose-urethane anesthesia with tracheal cannula connected with a light membrane valve through which various gas mixtures could be inhaled. Arterial blood pressure was recorded from a carotid or occasionally a femoral artery registering through a quick acting metallic membrane manometer. I am indebted to Prof. Velyien E. Henderson for this convenient instrument. Breathing movements were recorded by means of a tambour recorder applied to the thorax. Blood flow was observed by the use of a nickel wire indicator, heated by a constant current. Quick changes in blood flow, and the resultant alterations of electrical resistance in the nickel wire were indicated by the galvanometer in a Wheatstone bridge circuit. The needle carrying the nickel wire was about as large as 18

[1] I wish to thank Mr. F. A. MacDougal, superintendent of Algonquin Park, Ontario, for his kind and effective assistance in securing the beaver. Part of the expenses of the investigation were provided by a grant from the Penrose Fund of the American Philosophical Society.

gauge wire. It showed changes in flow rapidly, but did not indicate their magnitude in a quantitative manner.

The effect of CO_2 upon breathing. In conscious seals the addition of CO_2 to the air which was inspired through a mask frequently depressed the volume of pulmonary ventilation and did not regularly cause an increase until more than 5 per cent of CO_2 was present in the mask. When the CO_2 content reached about 10 per cent, ventilation was frequently only doubled, and the breathing was easy as compared with the rather violent hyperpnea which 10 per cent CO_2 causes in man and other laboratory animals (Irving, Solandt, Solandt and Fisher, 1935).

In about 25 observations on five beaver and six muskrats, no regular change in frequency of breathing with 10 per cent CO_2 appeared. Amplitude of breathing movements increased in every case, but the hyperpnea was never vigorous in comparison with the usual effect of 10 per cent CO_2 upon dogs, cats and rabbits.

The frequency of breathing of land animals often is not much affected by CO_2, but the amplitude and volume are easily increased. The degree of sensitivity is indicated by the observation of Haldane and Priestley (1905) to the effect that an increase of 0.2 volume per cent in the CO_2 of the alveolar air doubled the volume of human breathing. Unlike the ordinary mammals, the breathing of these diving animals is quite insensitive to CO_2.

The effect of CO_2 upon the heart and blood pressure. The frequency of the beaver's heart is subject to variations. Viewing many kymographic records of the heart beat of anesthetized beavers shows an irregularity of the interval between beats for which there was not obvious explanation although the number of beats per minute was regular. The frequency in four animals during chloralose-urethane anesthesia ranged between about 60 and 130, the lower rate characterizing more satisfactory preparations. Between one and three minutes after starting the inhalation of 10 per cent CO_2, when the amplitude of breathing had reached its maximum, the heart frequency had increased by 10 per minute only once in ten recorded observations on the four animals. In no instance did the arterial blood pressure change during the inhalation of CO_2.

In three recorded observations on two anesthetized muskrats (chloralose-urethane), the heart's frequency was between 340 and 390 and did not change for the inhalation of 10 per cent CO_2 during three or more minutes. The arterial blood pressure did not change.

Records of seven cats examined in the same way showed heart frequencies varying between 180 and 240 per minute while breathing air. In eighteen periods of inhalation of 10 per cent CO_2 for from one to ten minutes, the frequency of the heart changed in all but two instances. The changes varied between a decrease of 24 beats per minute and an

increase of 12 per minute. Ten experiments showed the decrease and six the increase, with the average and extreme increases smaller than the average and extreme decreases. The mean arterial blood pressure increased in every instance, the average of the increases being 12 mm. and the range extending between 3 and 24 mm. The amplitude of the heart beat record with a short period metallic membrane manometer was regularly increased by CO_2, and the pressure changes in the cardiac cycle were therefore increased, as Schneider and Truesdell (1922) observed in man.

The changes in cardiac activity which CO_2 produced in cats, and the regular increase in mean arterial pressure and in the difference between systolic and diastolic pressure indicate alterations in the circulation of the cat during the inhalation of CO_2. The lack of these changes in the beaver and muskrats, which was typical of a number of other incompletely recorded observations, suggests that the cardio-vascular system of the two diving animals is insensitive to CO_2. It is possible, however, that their circulation is not insensitive to CO_2, but rather that the vascular changes which occurred were so finely adjusted to each other as to leave the mean picture as recorded in heart frequency and blood pressure unchanged. The system of vascular control may present a picture of stability, when viewed through a resultant like the blood pressure, on account of perfect coördination of the heart and peripheral vessels, while the component parts of the vascular beds are actually considerably altered. In the latter case stability is maintained by sensitivity and fine coördination of the components, while on the basis of the former view, stability is due to insensitivity to CO_2. The following observations indicate that a large part of the peripheral circulation of the divers is like their breathing in being quite insensitive to CO_2.

Effect of CO_2 upon peripheral blood flow. Some of the earliest observations upon the control of the peripheral circulation indicated that inhalation of CO_2 caused peripheral vasoconstriction. Implications as to its effect upon blood flow were based upon the changes in blood pressure and upon observations of volume changes of tissues, but the conclusions were uncertain because of their indirect basis. Measurements of blood flow showed that the addition of CO_2 to the inspired air caused a decrease in blood flow through muscles of dogs (Keller, Loeser and Rein, 1930). The effect of inspired CO_2 in reducing muscular blood flow has been demonstrated in man (Lennox and Gibbs, 1932) and is regarded as common to mammals. That inhaled CO_2 does not modify blood flow through the muscles of muskrats and beaver is shown in the following section.

The effect of CO_2 upon blood flow through hind leg muscles (usually knee flexors) in a series of cats (chloralose-urethane anesthesia) is indicated in table 1. Among seven animals inhalation of 10 per cent CO_2 plainly diminished muscle flow in five, caused no change in one, and caused

increased flow in one. In one rabbit, flow also diminished. Observation of other muscles indicates that the behavior of the hind leg is typical. Numerous other observations have indicated that in about 8 out of 10 cases CO_2 consistently decreases muscle flow, but about 2 out of 10 animals have regularly failed to respond like the majority.

In men (Lennox and Gibbs, 1932) and in dogs (Irving and Welch, 1935) muscular blood flow is not decreased by the inhalation of CO_2 in a small proportion of individuals. Failure of the response is not uncommon, but we cannot suggest the conditions which distinguish the individuals in which it occurs, nor whether it represents an acute or chronic, local or general difference in their vascular control.

TABLE 1

Effect of inhalation of CO_2 and clamping the trachea upon blood flow in muscles

ANIMAL	DATE	10 PER CENT CO_2	CLAMP TRACHEA
Beaver	January 5, 1936	0	−
	March 28, 1937	0	−
	March 30, 1937	− (slight)	−
	March 31, 1937	0	−
Muskrat	April 15, 1936	0	−
	February 5, 1937	0	−
Cat	December 15, 1936	0	−
	January 15, 1937	−	−
	February 14, 1938	− (slight)	−
	February 21, 1938	+	+
	February 28, 1938	−	−
	March 23, 1938	− (slight)	−
	May 23, 1938	−	−
Rabbit	February 23, 1937	−	−

+, increased flow; −, decreased flow; 0, no change.

Keller, Loeser and Rein (1930) reported that the effect of CO_2 upon muscular flow was abolished by cutting the voluntary nerve supply. More specifically I have abolished the reduction of flow in a hind leg by excision of the lumbar sympathetic in five of these cats. Unilateral lumbar sympathectomy did not influence flow changes in the other leg. In three of the five sympathectomized cats, CO_2 inhalation was attended by increase of flow in the sympathectomized limb instead of the normal decrease.

In four beaver and two muskrats, inhalation of 10 per cent CO_2 caused a slight decrease in muscular blood flow in only one beaver. Muscles of the hind leg, neck and back have been examined and all behaved alike in this respect. Evidently CO_2 is not an effective stimulant for reducing

blood flow through muscle, just as it likewise fails to affect breathing, cardiac activity and arterial blood pressure.

The effect of clamping the trachea upon muscular blood flow. The arrest of breathing causes a reduction in flow through the muscles of cats, rabbits, dogs, muskrats and beaver (Irving, 1938). Table 1 shows that clamping the trachea reduced muscular blood flow in 6 of the 7 cats, in the muskrats, and in the three beaver which were examined (Irving, 1938).

The reduction in muscular blood flow during the arrest of breathing might have been caused by the accumulation of CO_2 in the cats, but not in the beaver and muskrats on account of their insensitivity. The diver's circulation is not generally insensitive, for in addition to the effect of arrested breathing, they showed strong pressor effects of carotid sinus stimulation and adrenaline.

The effect of CO_2 upon blood flow through the brain. The inhalation of CO_2 increases blood flow through the brain (Bronk and Gesell, 1927; Schmidt, 1928). In two beavers of this series it was observed that the inhalation of 10 per cent CO_2 increased blood flow through the brain (needle indicator inserted through trephined opening in the parietal eminence to a depth of 1 cm. into the cerebrum). As had been observed before (Irving, 1938) inflation of the lungs or any obstruction of breathing also increased cerebral flow. The effect of 10 per cent CO_2 upon cerebral flow was about the same in time and extent as the effect of arresting the breathing. In the sensitivity of the cerebral circulation of divers to carbon dioxide there is seen a contrast with the control of muscular flow.

Discussion and conclusions. According to the observations of Snyder and Rosenfeld (1937) the breathing movements of fetal mammals are not easily stimulated by CO_2, while after birth the typical adult sensitivity appears. On this basis it might be suggested that the insensitivity or tolerance of fetuses to CO_2 persists in the adult diver, but it does not seem likely that adult divers retain fetal characters.

Orr and Watson (1913) had reported that the breathing of ducks was not accelerated by CO_2, and Windle, Scharpenberg and Steele (1938) showed that after vagotomy CO_2 caused the ducks to breathe faster. They proposed that receptors must exist in the pulmonary field of the vagus which when stimulated by CO_2 caused an inhibition of breathing. The occurrence of chemoreceptors among the pulmonary vagus endings would be an important demonstration, but all respiratory adjustments, mechanical as well as chemical, are impaired by vagotomy, and so it does not seem justifiable on these grounds to attribute to the duck's vagus specific pulmonary receptors which inhibit breathing and which are sensitive to CO_2.

For several reasons it has seemed doubtful whether the vascular adjustment which follows the inhalation of CO_2 is really significant for preserva-

tion of an animal which is suddenly prevented from breathing. The change in muscular blood flow does not commence until about 30 seconds after the start of inhalation of 10 per cent CO_2 and then proceeds only slowly, so that its effect in preventing the muscles from the consumption of O_2 could not occur until the O_2 was already depleted. The failure of CO_2 to affect the muscular circulation in beaver and muskrats shows that these divers certainly do not utilize CO_2 as a stimulant for initiating this particular vascular adjustment.

The fact remains that the divers do successfully encounter asphyxial conditions which would be fatal to land animals. In case the reduction in muscular flow is necessary to the respiratory scheme of the divers, it can still be effected without sensitivity to CO_2; for divers are more effective than land animals in reducing muscular blood flow when breathing is arrested. The respiratory adjustments of diving animals are typically mammalian and differ only in a quantitative way from the adjustments of land animals. Since the internal vascular changes of divers are not affected by CO_2 it is fair to question whether CO_2 is really an important stimulus for quick adjustments in the internal respiration of mammals generally.

SUMMARY

The inhalation of CO_2 in concentrations up to 10 per cent does not cause much increase in breathing of the beaver and muskrat.

The changes in cardiac action and blood pressure which signify circulatory changes in the cat as a result of inhaling 10 per cent CO_2 do not appear in the muskrat and beaver. The general condition of the circulation of divers is not affected by 10 per cent CO_2.

The inhalation of CO_2, which usually causes a reduction in muscular blood flow in land mammals, has little effect upon muscular blood flow in the diving mammals. Since the arrest of breathing causes a similar decrease in muscular blood flow in diving and non diving mammals, CO_2 is probably not the stimulus which starts the reduction in muscle flow when breathing ceases.

REFERENCES

Bronk, D. W. and R. Gesell. This Journal **82:** 170, 1927.
Haldane, J. S. and J. G. Priestley. J. Physiol. **32:** 225, 1905.
Irving, L. This Journal **122:** 207, 1938.
Irving, L., O. M. Solandt, D. Y. Solandt and K. C. Fisher. J. Cell. and Comp. Physiol. **7:** 137, 1935.
Irving, L. and M. S. Welch. Quart. J. Exper. Physiol. **25:** 121, 1935.
Keller, C. J., A. Loeser and H. Rein. Ztschr. Biol. **90:** 260, 1930.
Lennox, W. G. and E. L. Gibbs. J. Clin. Invest. **11:** 1155, 1932.
Orr, J. B. and A. Watson. J. Physiol. **46:** 337, 1913.
Schmidt, C. F. This Journal **84:** 202, 1928.
Schneider, E. C. and D. Truesdell. This Journal **63:** 155, 1922.
Snyder, F. F. and M. Rosenfeld. This Journal **119:** 153, 1937.
Windle, W. F., L. G. Scharpenberg and A. G. Steele. This Journal **121:** 692, 1938.

THE EFFECT OF THE INHALATION OF GASES WITH A LOW OXYGEN AND AN INCREASED CARBON DIOXIDE-TENSION ON THE PERIPHERAL BLOOD FLOW IN MAN

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For an understanding of the effects of anoxia and carbon dioxide on the central nervous system (cf. Gellhorn, 1937) a study of the effects of changes in the tension of oxygen and carbon dioxide in the blood on the circulation is indispensable. Detailed investigations based on animal experimentation are reported elsewhere (Gellhorn and Lambert, 1938). Supplementary experiments on the human appear desirable and are reported below.

Aside from extensive studies on the effect of the inhalation of oxygen deficient gas mixtures and carbon dioxide on the blood flow through the brain (Cobb and Fremont-Smith, 1931; Lennox and Gibbs, 1932; Schmidt, 1934, and others) most studies carried out on the human subject were concerned with the reactivity of the blood pressure. They showed that the blood pressure is altered but little in normal young individuals (cf. E. Gellhorn, 1937). The peripheral flow was measured indirectly by Schneider and Sisco (1914) with Stewart's method (1911) in high altitude and showed an increase in perfusion rate. More recent and direct experiments by Freeman, Shaw and Snyder (1936) on the effect of oxygen deficiency on the peripheral blood flow showed variable results. In some cases an increase in perfusion rate, in others a decrease was found when the experimental subjects inhaled gas mixtures containing 7 to 15 per cent oxygen. In view of the irregularity of the results the reversal in blood flow after elimination of the sympathetic appears not convincing. Although extensive investigations have been published on the effect of carbon dioxide on peripheral blood vessels (Fleisch, Sibul and Ponomareo, 1932; Hermann, Morin and Vial, 1936) experiments on man have not been reported. We, therefore, chose to study the effect of various degrees of oxygen deficiency and carbon dioxide excess on the peripheral circulation by means of the plethysmographic method described by Freeman, with the modification that the

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temperature of the hand was kept constant by means of a temperature controlled water bath. The subject was allowed to rest for ½ hour in a reclined position and kept the hand within the plethysmograph for 15 minutes prior to the test so as to allow the circulation to become stable under 31°C. The hand within the apparatus rested on a wire mesh platform 1½ inches from the bottom. A blood pressure cuff was applied to the arm and the pressure was raised to 50 mm. Hg thereby occluding the venous outflow. During this interval the change in the hand volume was recorded on the kymograph by the bellows. Except in few instances all determinations were for 15 minute periods: 3 minutes control; 7 minutes inhalation of gas, and 5 minutes recovery. The volume of the hand was determined and the calculations recorded on the basis of blood flow per 100 cc. hand volume per minute.

RESULTS. The present study is based on 41 experiments carried out on 10 trained normal subjects. In 16 experiments 4–5.4 per cent carbon dioxide, in 25 tests 7.6–9.8 per cent oxygen was used.

The experiments with carbon dioxide gave uniform results (fig. 1) showing that during the inhalation of the gas mixtures the blood flow through the hand decreased regularly. Comparing the results of the effects of 4 per cent carbon dioxide with those obtained with the inhalation of 5.4 per cent carbon dioxide it may be said that they are similar but the degree of vasoconstriction was more marked in the latter group of experiments. Upon readmission of air the blood flow returned to its original level. It must be emphasized that in no case did a compensatory increase in blood flow occur although the reduction in blood flow during the period of carbon dioxide inhalation was frequently more than 50 per cent.

Far more complex are the results obtained with the inhalation of gas mixtures low in oxygen. Figure 2 shows some typical effects with 9.8 per cent oxygen. In all three experiments the blood flow is markedly increased during the period of anoxia and returns on readmission of air to its original level. In the experiment reproduced in the middle curve of figure 2 two anoxia experiments were performed in brief succession. The period of inhalation of 9.8 per cent oxygen was very short in the second experiment and did not lead to any change in blood flow but on readmission of air a greatly increased blood flow was observed.

More examples of such a compensatory hyperemia following a period of anoxia are shown in figure 3. The first two curves represent experiments in which 7.8 per cent oxygen was inhaled. Whereas during the period of anoxia the blood flow remained practically unchanged a marked increase in blood flow was observed after air had been readmitted. This compensatory increased blood flow lasted in the first experiment more than five minutes. In the last experiment of figure 3 in which 8.2 per cent oxygen was inhaled an increase in blood flow was observed during the period of

anoxia and the blood flow decreased gradually to the control level when air was readmitted. Nevertheless, this experiment shows a course which is somewhat different from that observed in the experiments of figure 2 in that a short period of reduced blood flow occurs during the initial period of anoxia before the blood flow increases distinctly above the control level.

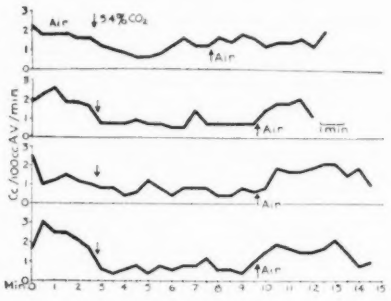

Fig. 1

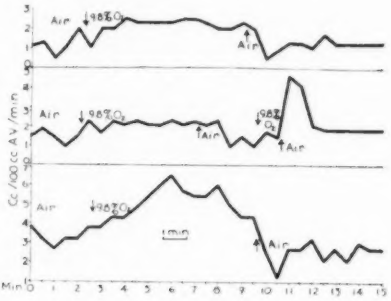

Fig. 2

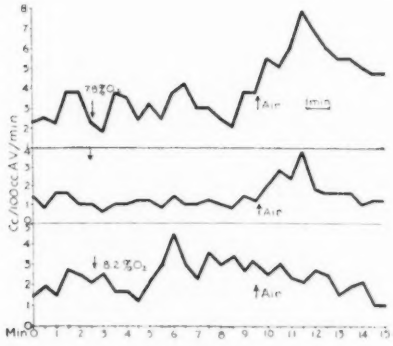

Fig. 3

Figs. 1–3. Records of the plethysmographic experiments. Ordinate = Blood flow in cubic centimeters per 100 cc. arm volume per minute. Abscissa = Time in minutes.

Fig. 1. Influence of 5.4 per cent carbon dioxide.

Fig. 2. Influence of 9.8 per cent oxygen.

Fig. 3. The upper two graphs show the effect of 7.8 per cent oxygen; the last graph that of 8.2 per cent oxygen.

The results described thus far indicate that the effects of oxygen on the peripheral blood flow in the human may be of two different types. One finds either an increased blood flow during the period of anoxia which is followed by a normal blood flow in air, or the blood flow remains un-

changed during anoxia and is followed by a marked compensatory phase on readmission of air during which period the blood flow is increased.

Figures 2 and 3 suggest that the type of response observed depends largely on the oxygen concentration inhaled. This is borne out in figure 4 in which a summary of all anoxia experiments is given. The experiments are divided into two groups of about the same size. The first comprises 12 experiments with 9.8 per cent oxygen and the second 13 experiments with 7.6–8.4 per cent oxygen. Whereas in the first group the subjective

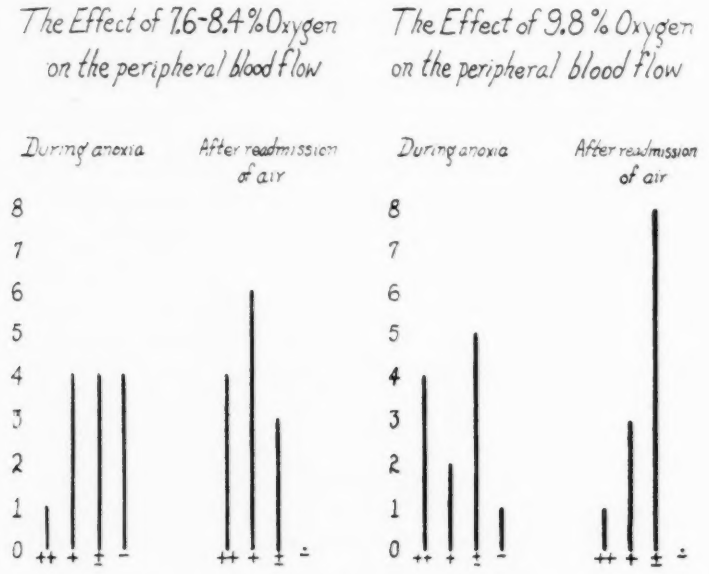

Fig. 4. Distribution of the various effects of oxygen deficiency during and after the administration of oxygen deficient gas mixtures. +, ++ = moderate and marked increase in blood flow respectively. ± = no change in blood flow. − = decrease in blood flow. Ordinate: number of cases.

effects induced by the inhalation of a gas mixture with a reduced oxygen tension are small or completely absent definite symptoms result regularly from the inhalation of the gas mixtures used in the second group of experiments. The figure indicates clearly that although both types of response are observed with either oxygen concentration the marked increase in blood flow occurs predominantly when the higher oxygen concentration is inhaled. Moreover, a decrease in blood flow during the anoxia period occurred only in one experiment when 9.8 per cent oxygen was inhaled but was observed four times when 7.6–8.4 per cent oxygen was used.

With this distribution of the effects agree the changes in blood flow observed on readmission of air. In the experiments carried out with 9.8 per cent oxygen the blood flow was unchanged in the majority of cases during the second control period whereas in the group with more severe oxygen deficiency the compensatory increase in blood flow predominates.

The results are easily understood on the basis that the blood flow through the hand under conditions of oxygen want is determined largely by two factors:

1. The general vasoconstrictor effect leading to an increased blood pressure.

2. The peripheral dilatory effect of oxygen deficiency due to accumulation of metabolites which is very marked in animal experiments in which the sympathetic effects have been eliminated (Herman, Morin and Vial, 1936).

Under physiological conditions the blood flow will be determined by the balance of the two forces. Our results indicate that with increasing severity of the anoxia the general vasoconstrictor effect becomes increasingly effective. This is shown not only in the relatively large number of cases in which an actual reduction of blood flow was observed during severe anoxia (cf. fig. 4) but also by the fact that when the blood flow increased during the period of oxygen it occurred relatively infrequently and to a moderate degree. On the other hand, in the experiments with moderate anoxia (9.8 per cent oxygen) the general vasoconstrictor effect was so weak that marked increase in blood flow frequently occurred.

The behavior of the blood flow on readmission of air is solely determined by the actual oxygen want of the tissues. The general vasoconstrictor effect quickly subsides as can be seen from continuous blood pressure records (Darrow and Steck). Since in the experiments with severe anoxia the blood flow was at best only moderately increased during the oxygen deficiency period the tissues are in a state of oxygen debt which is repaid by an increased blood flow on readmission of air. In the experiments with moderate anoxia the blood flow was predominately increased during the period of oxygen want and returned in most cases to the control level on readmission of air indicating that the increased blood flow had fully compensated for the decreased oxygen tension of the blood.

That the increased respiration is not responsible for the results described is evident, since in the experiments with carbon dioxide the respiration was greatly increased but the results were fundamentally different from those observed in anoxia.

Figure 4 shows clearly that the explanation given is adequate but it also indicates that almost any type of response may be obtained in anoxia. The strength of the sympathetic reaction is individually different for a given oxygen concentration. It must depend on the degree of respiratory

adjustment, the increase in minute volume of the heart and other factors (cf. for further discussion, Gellhorn and Lambert, 1938). The local response due to metabolites is also dependent on the degree of circulatory and respiratory adjustment. For these reasons a constant reaction of the respiratory blood flow to anoxia is not to be expected. Therefore, the study of the peripheral blood flow under the influence of a reduced oxygen tension in the blood is not a suitable test for the presence or absence of sympathetic impulses as Freeman (1936) suggested.

The situation is, however, different with regard to carbon dioxide. Our experiments have clearly shown that the inhalation of 4–5 per cent carbon dioxide in air regularly leads to a decrease in peripheral blood flow. On readmission of air the original blood flow is restored. Obviously the blood flow in this group of experiments is solely determined by sympathetic impulses concealing completely the local effects on the blood vessels which in the absence of central nervous impulses may lead to a distinct vasodilatation and increased blood flow.

The expectation that such an effect (increased blood flow) may result from the inhalation of 5 per cent carbon dioxide in the sympathectomized arm has been verified in numerous tests (Steck and Gellhorn) and will be discussed elsewhere.

In spite of the differences observed between the experiments with anoxia and those with carbon dioxide it is worthy of mention that not only in the experiments involving oxygen want but also in those with carbon dioxide the amount of oxygen supplied to the tissues is reduced. This leads to the compensatory blood flow either during the anoxia period or on readmission of air in the experiments involving oxygen deficiency. Although the oxygen tension is not appreciably altered in the carbon dioxide experiments the great reduction in blood flow probably leads to a decreased oxygen supply to the tissues. This may in part be counteracted by the shift in the dissociation curve of oxygen hemoglobin to the right (Barcroft). Another possibility is that carbon dioxide reduces the tissue metabolism. This has recently been shown to be true by Rein. Experiments of Rein (1936) and Kramer (1937) with the thermo-stromuhr show that in narcotized animals anoxia is followed by a compensatory increase in blood flow whereas it is absent after carbon dioxide. The results fully agree with our experiments in man involving an entirely different method.

SUMMARY AND CONCLUSIONS

By means of the plethysmographic method described by Freeman a series of experiments on the influence of the inhalation of gas mixtures with a reduced oxygen tension or an increased carbon dioxide tension on the blood flow in the human hand were carried out.

It was found that carbon dioxide in air (4–5.4 per cent) regularly reduces the blood flow. On readmission of air the blood flow rises to the original control level. A compensatory phase is conspicuously absent.

Inhalation of gas mixtures containing 7.6–9.8 per cent oxygen gave variable results. In the milder degree of oxygen lack (9.8 per cent) the response to oxygen lack was predominantly an increased blood flow whereas with lower oxygen concentrations the blood flow was either decreased, unchanged or slightly increased. On readmission of air a compensatory blood flow was observed in most cases. The variability of the results is due to the fact that the central sympathetic impulses compete with the dilatory effect of local metabolites.

The regularity of the results obtained with carbon dioxide make these experiments a more suitable test for the presence or absence of sympathetic impulses rather than experiments involving oxygen want.

REFERENCES

BARCROFT, J. Hemoglobin. Cambridge, 1928.

COBB, AND S. F. FREMONT-SMITH. Arch. Neurol. and Psychiat. **26:** 731, 1931.

DARROW, C. AND I. E. STECK. Unpublished observations.

FLEISCH, A., I. SIBUL AND V. PONOMAREV. Pflüger's Arch. **230:** 814, 1932.

FREEMAN, N. E. This Journal **113:** 384, 1935.

FREEMAN, N. E., J. L. SHAW AND J. C. SNYDER. J. Clin. Invest. **15:** 651, 1936.

GELLHORN, E. Ann. Intern. Med. **10:** 1267, 1937.
Sigma Xi Quart. **25:** 156, 1937.

GELLHORN, E. AND E. LAMBERT. The vasomotor system in anoxia and asphyxia. Medical Monograph Series, Univ. of Illinois Press, 1938. In press.

HERMAN, H., S. MORIN AND J. VIAL. Annal. de physiol. **12:** 255, 1936.

KRAMER, K. Pflüger's Arch. **238:** 91, 1937.

LENNOX, W. G. AND E. L. GIBBS. J. Clin. Invest. **11:** 1155, 1932.

REIN, H. Nachr. Ges. Wiss. Göttingen. Math.-Physik. Kl. **2:** no. 14, 1936.

SCHMIDT, C. F. This Journal **114:** 572, 1936.

SCHNEIDER, E. C. AND D. L. SISCO. This Journal **34:** 29, 1914.

STECK, I. E. AND E. GELLHORN. In preparation.

STEWART, G. N. Heart **3:** 33, 1911.

HYPERTHYROIDISM AND CARDIAC GLYCOGEN[1]

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It appears to be generally admitted that the ill effects of thyroid substance upon the heart are functional rather than structural. Interest in the nutritional state of the heart in hyperthyroidism is great. Many observations concerning cardiac metabolism in this condition that appear in the literature are couched in terms too general for critical evaluation; many appear inadequately supported by experimental work. During the course of a study of the hyperthyroid heart in this laboratory, we have offered definite figures for: the oxygen consumption and the effect of epinephrine upon the heart in a state of extreme hyperthyroidism (McDonald et al. 1935) and the work done, the oxygen consumed, and the behavior toward epinephrine by the heart in a state of moderate hyperthyroidism (McDonald, 1937).

Cardiac glycogen has been the subject of numerous investigations conducted upon the hyperthyroid heart. Andrus (1932) found the isolated auricles of hyperthyroid rabbits to have a definitely lowered glycogen content. Hoet and Marks (1926) describe the hearts of hyperthyroid rabbits as totally free of glycogen. Lederer (1936) reports that he has completely cleared the heart of glycogen and caused the appearance of cardiac disturbances by the injection of thyreotropic hormone.

The exact rôle played by glycogen in the heart's metabolism is by no means definitely known. Lederer states that a critical level for glycogen is necessary to the heart's normal functioning and that when it falls below this level, disturbances appear. In the isolated heart the performance of work is known to be attended by a decrease in the store of glycogen (Visscher and Mulder, 1930). Clark and his co-workers (1931) have offered evidence that over 70 per cent of cardiac metabolism is normally other than carbohydrate. Wertheimer (1933) states that only after a definite load limit for cardiac muscle has been passed, that is a maximal reserve limit expressed in terms of initial length of fiber has been exceeded, is there a call upon carbohydrate for energy purposes.

The series of experiments described herein was planned to study the

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effect upon glycogen content of the several parts of the heart as the hyperthyroidism developed. Dogs were chosen as the experimental animals because they readily adapt themselves to laboratory procedure and do not after training show an accelerated heart rate when handled as do rabbits and other small laboratory animals. The tachycardia of hyperthyroidism in dogs is not stable and may suddenly give place to a normal rate even though the administration of thyroid substance is not diminished. We regarded the difficulty of maintaining a tachycardia as a lesser source of potential error, however, than the reflex acceleration so readily shown by other animals.

METHODS. Dogs free of disease and in a good state of nutrition were isolated and placed upon a diet adequate in the known food requirements. This diet was varied regularly by using three different basic diets. The dogs were taught to lie quietly upon a table while repeated electrocardiographic observations were made to establish a basal heart rate for each dog. Thyroid substance[2] was then given by mouth in the daily dosage of 0.4 gram per kilo of body weight until the heart rate indicated the development of a hyperthyroid state. The dogs were selected for study after varying periods of tachycardia.

The animal was brought to the laboratory, weighed, and placed upon the operating table as quietly as possible by his regular attendant. Sodium phenobarbital solution (50 mgm. to the cc.) was injected into the saphenous vein in sufficient dosage to produce surgical anesthesia rapidly. From 100 to 150 mgm. per kilogram of body weight constituted the dosage. The average time for the induction of surgical anesthesia was from 4 to 5 minutes. The chest was opened and the heart, still beating, was removed and divided into the two atria, the two ventricles, and the septum. A piece of each division was excised, dropped into a weighed hydrolyzing tube, and weighed. Hot hydrolyzing fluid (30 per cent KOH solution) was then added. The whole procedure from opening the chest to this point consumed less than 5 minutes.

The sites chosen for excision of the tissues for hydrolysis were: atria, practically all that remained after the septum and auricle were removed; right and left ventricles and septum, a transverse piece from near the middle (that from the left ventricle was slightly nearer the base than the apex).

Hydrolysis of the tissue was carried out according to Pflüger's method as modified by Good, Kramer, and Somogyi (1933). We made two further slight modifications: 1, the tissue was weighed before the hydrolyzing fluid was added, and 2, following acid hydrolysis of the glycogen, the solution was made neutral to litmus paper by the addition of NaOH solution.

[2] Thyroid substance furnished through courtesy of Eli Lilly & Co.

TABLE 1

Basal heart rate, highest rate, and the time required to establish a definite tachycardia

DOG NUMBER	BASAL RATE	HIGHEST RATE	DAYS REQUIRED
1	66	175	20
2	55	130*	21
3	60	120	11
4	75	130*	15
5	88	159	18
6	69	120	17
7	85	152	14
8-A	81	180	33

* Higher rates were obtained in these animals by increasing the dosage of thyroid substance.

TABLE 2

Glycogen content of control group of dogs

(mgm. per gram of tissue calculated as glucose)

NUMBER	ATRIA		VENTRICLES		SEPTUM
	Right	Left	Right	Left	
N-1	4.44	5.53	6.98	4.29	6.63
N-2	6.67	9.37	10.42	6.71	7.15
N-4	5.60	6.84	7.78	6.57	5.98
N-5	6.13	7.42	6.95	5.75	4.85
N-6	5.90	8.89	10.33	7.50	7.19
N-7	5.27	5.05	5.82	4.30	4.70
N-8	4.65	5.01	6.03	6.81	6.63
Average.....	5.53	6.89	7.76	6.00	6.16

TABLE 3

Glycogen distribution in the hearts of hyperthyroid dogs showing a tachycardia of short duration

DOG NUMBER	DURATION OF TACHYCARDIA	PERCENTAGE OF WEIGHT LOSS	GLYCOGEN EXPRESSED AS MGM. GLUCOSE PER GRAM OF TISSUE				
			Atria		Ventricles		Septum
			Right	Left	Right	Left	
	days						
3	3	21	4.20	4.77	6.62	4.46	5.16
6	10	20	5.19	5.33	6.55	4.11	4.71
1	15	19		7.89	7.95	7.33	8.39

Glucose was determined by the method described by Shaffer and Somogyi (1933), using the modified reagent no. 50 and the modified procedure suggested by Somogyi (1937).

RESULTS. Two hyperthyroid dogs and a normal dog were each given epinephrine intravenously. The drug was administered in from 0.5 to 1 mgm. at 20 minute intervals. Dog 2 died during the injection of the first

TABLE 4

Glycogen distribution in the hearts of hyperthyroid dogs showing a tachycardia of long duration

DOG NUMBER	DURATION OF TACHYCARDIA	PERCENTAGE OF WEIGHT LOSS	GLYCOGEN EXPRESSED AS MGM. GLUCOSE PER GRAM OF TISSUE				
			Atria		Ventricles		Septum
			Right	Left	Right	Left	
	days						
7	41	37	2.38	3.09		3.23	3.60
4	47*	23	4.67	8.47	6.08	3.78	2.85

* Daily dosage of thyroid substance increased from 0.4 to 2 grams per kilogram of body weight for last 11 days.

TABLE 5

The effects of epinephrine upon the glycogen distribution in the hearts of hyperthyroid and normal dogs

DOG NUMBER	DURATION OF TACHYCARDIA	PERCENTAGE OF WEIGHT LOSS	GLYCOGEN EXPRESSED AS MGM. GLUCOSE PER GRAM OF TISSUE				
			Atria		Ventricles		Septum
			Right	Left	Right	Left	
	days						
5	46	21	2.55	3.75	5.00	2.68	2.29
2	69	00	3.13	5.59	5.31	2.71	2.21
8-D	00	00	3.60	5.25	6.09	5.23	4.87

TABLE 6

The effects of glucose-insulin-epinephrine on the glycogen distribution in the hearts of hyperthyroid and normal dogs

DOG NUMBER	DURATION OF TACHYCARDIA	PERCENTAGE OF WEIGHT LOSS	GLYCOGEN EXPRESSED AS MGM. GLUCOSE PER GRAM OF TISSUE				
			Atria		Ventricles		Septum
			Right	Left	Right	Left	
	days						
8-A	34	16	2.50	1.30	2.06	1.82	0.79
7-D	00	00	4.04	9.64	4.34	1.28	3.30

0.5 mgm. Dog 5, hyperthyroid, and dog 8-D, normal, each received 4.5 mgm. of epinephrine. The glycogen content of these hearts is shown in table 5.

In an effort to see whether insulin might protect the hyperthyroid heart against loss of glycogen when treated with epinephrine, 1 hyperthyroid dog and 1 normal dog were given glucose (20 grams), insulin (10 units),[3] and epinephrine (4.5 mgm.). Table 6 expresses the results.

DISCUSSION. In the consideration of any data derived from an experimental hyperthyroidism, it is well to keep in mind that such hyperthyroidism is not identical in every way with the clinical entity. For example, our dogs showed few symptoms of nervousness and no muscular weakness. Diarrhea gave us but little trouble. Most of our dogs lost weight with moderate severity; a few actually gained in weight. We believe that our practice of rotating the several basic diets was a factor in keeping our animals in a good state of nutrition. Carlson (1932) has shown that the contractions of the empty stomach in hyperthyroidism differ in type and amplitude from the normal, the emptying time of the stomach is decreased, and the speed of a barium meal through the small intestines is increased. The questions may well arise: 1, how much of the loss in weight in hyperthyroidism is due to starvation because of the rapid progress of food through the alimentary canal rather than to the specific effects of the thyroid secretion upon cellular metabolism, and 2, to what extent is the diarrhea dependent upon incomplete digestion and absorption rather than to hyperirritability of the gut.

Weight changes, either for loss or gain, often occurred very rapidly, sometimes involving a kilogram a day. Coincident with rapid weight loss we noted on numerous occasions the development of a paroxysmal tachycardia of atrial origin. This seemingly had no connection with the length of time the animal had been on thyroid substance. It disappeared when the animal began to gain in weight again. Illustration 1 shows the record derived from lead II of the electrocardiograph during a period of tachycardia and after recovery therefrom.

Analysis of one heart during such a paroxysmal attack showed all of its parts to be rich in glycogen (dog 1, table 3). Undoubtedly the onset of this cardiac disturbance is not dependent upon an exhaustion of myocardial glycogen. The heart of this dog responded with this behavior to some factor associated with general well-being which is not reflected in a decrease in cardiac glycogen. Consequently, one may wonder what part of the cardiac disturbances of both experimental thyreotoxicosis and of Graves' disease is nutritional rather than the direct effect of the thyroid secretion. The clinical implications of this idea need no elaboration.

As may be noted in table 3, no significant changes in the glycogen content of the hearts in this series occurred in short periods of tachycardia. Dog 1, with a tachycardia of 15 days' duration and characterized by the

[3] Insulin furnished through courtesy of Eli Lilly & Co.

paroxysmal behavior just described, showed a glycogen content in all parts of the heart quite comparable to that of the average normal heart.

Longer periods of tachycardia (table 4) were characterized by substantial losses in glycogen, particularly in the left ventricle and the septum wherein the glycogen content fell materially below the minimal value found for normal hearts. In no case, however, could we describe any part of the heart as being "totally" free of glycogen.

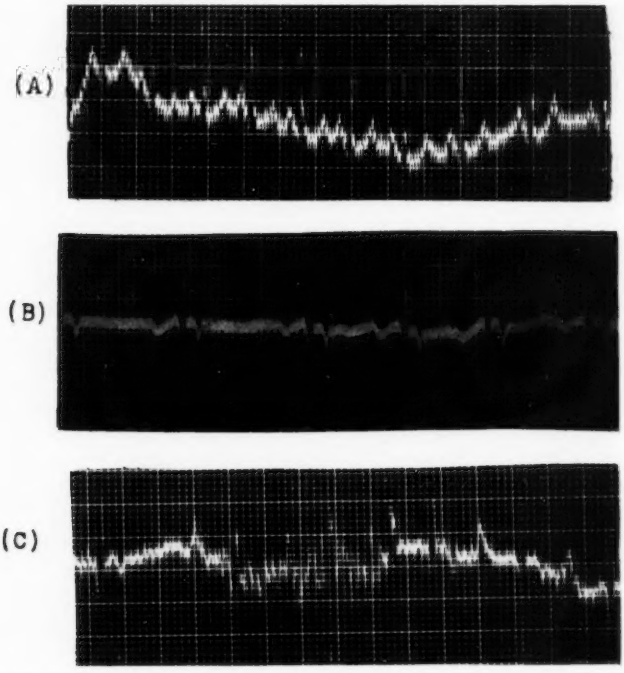

Fig. 1. (*A*) A period of paroxysmal tachycardia coincident with rapid weight loss. The rate, counted by the "R" wave over a long strip, was 198. (*B*) The rate characteristic to the animal at this time. Computed in the same manner it was 108 per minute (*C*) Shows the sudden onset and cessation of a paroxysm.

One of us has noted that the hearts of hyperthyroid terrapins show less endurance when exposed to the action of epinephrine when compared to normal hearts. We wished to determine if this were true for the dog's heart and, if so, whether it was related to the glycogen content. Table 5 shows the results of the administration of epinephrine. Such experiments gave us our lowest glycogen values, yet in no instance was any part of the heart wholly free of glycogen. Particularly interesting was the case of

dog 2 (table 5) which died from cardiac failure during the injection of the first 0.5 mgm. of epinephrine. The heart, after a momentary flutter, stopped in diastole. Complete arrest was confirmed by the electrocardiograph. In dog 5 there was a progressive reduction in the response of the heart to repeated doses of epinephrine which offers further analogy to the behavior of the hyperthyroid terrapin's heart. Insulin plus glucose failed to protect the hyperthyroid heart against loss of glycogen following the administration of epinephrine.

Routine paraffin, H. & E. sections were made of all chambers of these hearts. The changes were mild and purely alterative. In two dogs acute, exudative lesions were believed to be unrelated to the hyperthyroidism.

SUMMARY

1. The development of cardiac irregularities in hyperthyroidism is not primarily dependent upon the glycogen exhaustion of any part of the heart and may occur in a heart whose parts exhibit a rich supply of glycogen.

2. Short periods of simple tachycardia do not alter the heart's store of glycogen. Longer periods cause some reduction. Only in the left ventricle and the septum did we find this reduction substantial in amount.

3. Extreme degrees of irregularity may appear coincident with evidence of marked systemic disturbance as indicated by rapid loss in weight and may disappear with a resumption of gain in weight.

4. Epinephrine shows a much greater ability to decrease the heart's store of glycogen in the hyperthyroid heart than in the normal heart. Insulin and glucose fail to offer protection against this decrease.

5. Hyperthyroidism reduces the capacity of the heart to respond to epinephrine. With this goes a reduction in the glycogen content of the myocardium which is most marked in the left ventricle and the septum.

6. From this work and that previously published, we wish to offer the view that the cardiac failure of hyperthyroidism is not contingent upon the presence or absence of glycogen and that the function of cardiac glycogen is not primarily to produce energy but, in some manner unknown to us, to act as a stabilizer between the conservation of energy and its expenditure.

7. Pathological changes in the myocardium were of the most minor character, namely, parenchymatous degeneration.

REFERENCES

ANDRUS, E. C. Am. Heart J. **8:** 66, 1932.

CLARK, A. J., R. GADDIE AND C. P. STEWARD. J. Physiol. **72:** 443, 1931.

FETTER, D. AND A. J. CARLSON. This Journal **101:** 598, 1932.

GOOD, C. A., H. KRAMER AND M. SOMOGYI. J. Biol. Chem. **100:** 485, 1933.
HOET, J. P. AND H. P. MARKS. Proc. Roy. Soc. B. **100:** 72, 1926.
LEDERER, J. A. Paris Medical **26:** 25, 1936.
McDONALD C. H., W. L. SHEPEARD, M. F. GREEN AND A. F. DEGROAT. This Journal **112:** 227, 1935.
McDONALD, C. H. Am. Heart J. **13:** 687, 1937.
SHAFFER, P. A. AND M. SOMOGYI. J. Biol. Chem. **100:** 695, 1933.
SOMOGYI, M. Ibid. **117:** 771, 1937.
VISSCHER, M. B. AND A. G. MULDER. This Journal **94:** 630, 1930.
WERTHEIMER, E. Pflüger's Arch. **232:** 120, 1933.

FURTHER STUDIES ON ZINC DEFICIENCY IN RATS[1]

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The basal ration which has been used in this laboratory for the demonstration of the essential nature of zinc in the nutrition of the rat has never given a consistent rate of growth greater than 2.4 grams per day during the first six week period, when adequate zinc was supplied. This would indicate that although every attempt was made to include all essential nutrients some of them were not present in adequate amounts. Another difficulty encountered was the use of milk serum which showed considerable seasonal variation in growth-promoting factors. For these reasons we wish to describe the results obtained when a liver extract, rendered low in zinc by extraction with dithizone, was substituted for the milk serum.

Analysis of tissues and whole carcasses of zinc deficient rats and added zinc controls are reported in the present paper. We also wish to record our results on the relation of zinc to pancreatic enzymes, especially the proteolytic enzymes.

EXPERIMENTAL. Young rats at two weeks of age, and their mothers, were placed in a monel metal, screen bottom cage. The mother was removed twice daily for separate feeding. At twenty-one days of age the young were housed in individual monel metal cages and placed upon the following ration.

Sucrose	68.0 parts
Casein	5.0
Egg white	14.0
Salts	4.0
Peanut oil (commercial)	4.0
Butter fat (+ 1 gram haliver oil per kilo)	0.5 gram/day
Vitamin B_1	6.0 micrograms per day
Purified liver extract	1.0 cc. per day

In a previous paper (1) it has been shown that 42 micrograms of zinc per day per rat is just sufficient to allow growth at a rate comparable to

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that of controls receiving 300 micrograms of zinc per day. In the studies reported here, control rats were given 100 micrograms of zinc (as the sulfate) per day to insure an optimum amount in view of the more rapid growth rate on the present ration. The casein, egg white, salts and butter fat were prepared as described in a previous paper (2). Nickel and cadmium (as the sulfates) were added to the salt mixture at a level of 5 mgm. per 100 grams. The sucrose was thrice recrystallized from 80 per cent alcohol. This treatment reduced the zinc content from 11 mgm. per kilo to 0.46 mgm. per kilo of sucrose. The complete dry ration analyzed 0.92 mgm. zinc per kilo. This allows about 5 micrograms zinc per 5 grams of ration, the approximate daily intake.

TABLE 1

Weekly growth averages of male rats on new zinc ration

WEEK ON EXPERIMENT	100 MICROGRAMS ZINC/DAY		ZINC LOW	
	Number of rats	Average weekly weight change	Number of rats	Average weekly change in weight
		grams		*grams*
1	8	12.6	16	13.5
2	8	17.3	16	8.9
3	8	27.5	16	9.1
4	8	26.8	16	6.4
5	8	27.0	16	8.8
6	6	25.0	13	5.5
7	5	24.0	11	6.3
8	3	15.0	3	3.0
9	2	12.0	3	5.0
10	2	12.0	3	9.0

The zinc determinations have been made according to the dithizone-iodometric method of Sylvester and Hughes (3). By doubling the dilution of the thiosulfate this method is sensitive to about 20 micrograms of zinc. Recoveries of added zinc are quantitative.

The liver product which we have used in the above rations was a 92 per cent alcohol soluble material,[2] although any potent liver extract could be utilized with equal success. Twenty grams of this material were made up to 100 cc. with distilled water, the insoluble material filtered off, and the filtrate (pH = 3.8) extracted with 30 cc. lots of a 0.1 per cent solution of dithizone (diphenyl thiocarbazone) in carbon tetrachloride (distilled). Extractions were repeated until the dithizone-carbon tetrachloride phase no longer showed discoloration; about 200 cc. of the solution are required. The dye may be recovered by washing in 1 N HCl. The purified liver

[2] We are indebted to Dr. D. Klein, Wilson Laboratories, Chicago, for a generous supply of this liver produce.

extract is left under vacuum overnight to remove dissolved CCl_4, and a little purified sucrose is added to make it more palatable. The zinc content of the liver extract was reduced from 158 mgm. per kilo to 7.41 mgm. per kilo by this process; therefore, the daily dose of 1 cc. contains 1.6

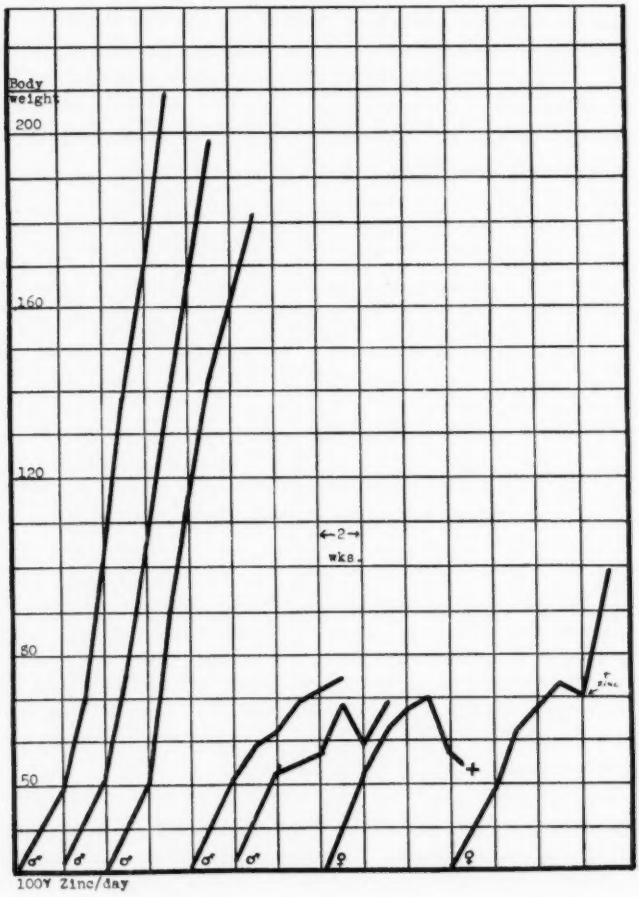

Chart 1. Growth record of litter mates on new zinc ration

micrograms of zinc. The total daily zinc intake on the above diet (dry ration plus liver extract) is about 7 micrograms.

Table 1 gives the growth records of the male rats on the above (zinc low and added zinc) rations. The individual growth records of one litter are shown in chart 1. This effect of zinc on growth is apparently specific,

for the trace elements Fe, Mg, Mn, Cu, I, Ni and Cd, are included in the salt mixture, and analysis of the ration shows it to have a higher Al, As and Co content than mineralized milk (dry basis).

Tissue analysis. The zinc content (dry basis) of the whole carcass and the various tissues of zinc deficient rats (65–80 grams) and added zinc controls (120–140 grams) are given in table 2. The GI tract of the rats was removed prior to analysis of the whole carcass in order that the results would not be confused by the presence of the extraneous food materials

TABLE 2

Zinc content of tissues of zinc deficient rats and added zinc controls

TISSUE	ADDED ZINC RATS 100 MICROGRAMS ZINC/DAY	ZINC DEFICIENT RATS
	mgm. Zn./k. (dry)	*mgm. Zn./k. (dry)*
Whole rats (GI tract removed)	65.3	51.0
	77.3	57.2
Muscle	44.7*	45.2*
	45.1	47.7
Skin + hair	53.6*	64.0
	64.9	61.3
Liver	79.1	55.9
	72.8	70.0
	76.0	68.2
Small intestines	112.0	85.0
	114.3	90.2
Blood: mgm./100 cc.	0.60	0.25
	0.72	0.20*
Bone	90.8*	62.1*
	93.2	61.9*
		63.0
Teeth	91.8*	49.1*
Daily zinc excretion in feces	45 micrograms	3 micrograms
	51 micrograms	2 micrograms
	40 micrograms	4 micrograms

* Composite samples from 2 rats.

Figures for whole rats are averages of triplicates; others are single determinations.

in the tract. Feces were collected for a three-day period and analyzed for zinc. These results, in terms of zinc excreted per day through the feces, are included in table 2.

The percentage of dry matter of the whole rat and tissues is given in table 3.

Relation of zinc to pancreatic enzymes. Previous work (1) has shown that a marked delay in the rate of absorption from the intestines is associated with the zinc deficiency. Intestinal stasis is a secondary symptom

common to many nutritional deficiencies and it is possible that this may be due, in part at least, to a slower rate of hydrolysis of food components by enzymes in the tract. To test this hypothesis we have determined the activity of pancreatic enzymes of zinc deficient rats as compared with the added zinc controls.

TABLE 3

Dry matter of zinc deficient and added zinc rats

TISSUE	ADDED ZINC RATS PER CENT DRY MATTER	ZINC DEFICIENT RATS PER CENT DRY MATTER
Whole rat	31.9	30.3
	31.1	29.5
Muscle	26.2	25.5
Bone (ash)	65.5	64.6
	66.4	65.5
	64.2	65.0
Liver	28.0	26.9
	29.3	25.0
	30.3	31.3
Pancreas	38.7	32.6
	36.4	32.4
	30.9	28.1

TABLE 4

Pancreatic proteolytic activity of male rats under various conditions

	NUMBER OF ANIMALS	RANGE	AVERAGE
		cc. N/50 KOH	*cc. N/50 KOH*
Added zinc rats	6	6.38- 9.14	7.70
Zinc deficient rats	8	1.35- 6.72	4.24
Rats on magnesium deficient ration	4	2.90- 6.35	4.82
Stock rats:			
(1) 70 grams	3	8.56- 9.29	8.89
(2) 200 grams	3	7.00- 8.65	7.94
(3) Starved 6 days	3	9.80-12.45	11.50
(4) Starved 6 days + 4 hrs. on feed	3	6.62- 7.30	7.08
(5) Starved 6 days + 6 days on feed	4	6.00- 8.45	7.85
(6) High zinc (1 per cent) ration	4	7.40- 8.75	7.90

The pancreas was removed from the animal and homogenized in sufficient 0.01 N HCl to make a final concentration of 50 mgm. of tissue per cubic centimeter (final pH = 4.1).

For proteinase activity determination this suspension was allowed to stand for 24 hours to permit spontaneous activation of the tryptic enzymes. One cubic centimeter portions were then added to 5 cc. of a 2.5 per cent purified casein solution (disodium phosphate buffer, pH = 8.2).

The rate of hydrolysis was followed by the formol titration, and activity expressed as the cubic centimeter of N/50 KOH required (phenolphthalein) after a hydrolysis period of 9 hours at 37°C. The proteolytic activity of zinc deficient rats was found to be lower than that of added zinc rats (table 4).

This effect suggested, at first, that zinc may function either as a specific activator of tryptic enzymes or that it is involved in the spontaneous conversion of trypsinogen into the tryptic enzymes. Neither of these possibilities is plausible in view of the fact that the zinc concentration of the tissues of zinc deficient rats is not markedly lower than that of the added zinc controls. (However, the pancreas could not be analyzed for zinc because of its small size.) The first possibility was definitely disproved when 5 micrograms of zinc (as sulfate) added to the casein solution were

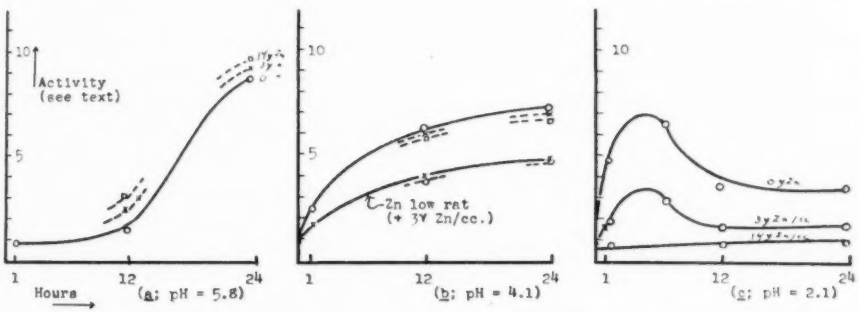

Chart 2. Activation rates of normal pancreas tryptic enzymes at various pH levels and in presence of various levels of zinc ion. The lower curve in (*b*) represents the activation rate of pancreas of a zinc deficient rat with and without in vitro zinc additions (3 micrograms zinc per cc.).

shown to have no influence on the rate of hydrolysis by the zinc deficient pancreas. The second possibility has also been eliminated since zinc sulfate, when added to the pancreatic suspension immediately after removal of the pancreas from the animal, has no effect on the rate of spontaneous activation of the tryptic enzyme (chart 2 b).

The proteolytic activity of the pancreas of rats in a severe state of malnutrition on a magnesium low ration has also been found to be lower than normal (table 4), indicating that this effect is not specific for any one deficiency. The observed lowering of activity is not due merely to the smaller body size of the deficient rat, for young stock rats (70 grams) have fully as great pancreatic proteolytic activity as adult stock rats (200 grams) (table 4). Neither can the effect be explained as an artifact due to lessened food consumption, for starvation of stock rats resulted in a higher enzyme content of the pancreas (table 4).

The effect of high amounts of zinc on the rate of spontaneous activation of the pancreatic tryptic enzymes of adult stock males has also been studied (chart 2 a, b, c). The pancreases from 6 normal (stock) male adults were pooled and minced. Aliquots were weighed into 9 test tubes and brought to a concentration of 50 mgm./cc. with the appropriate dilute HCl solutions having the indicated zinc content. The activity at various times during the 24 hours was determined by taking 1 cc. aliquots, adding these to 5 cc. of a buffered casein solution and determining the volume of N/50 KOH required after incubation for 9 hours at 37°C. The zinc ion has a definite inhibitory effect when activation is allowed to proceed at a pH of 2.1, but practically no effect at pH 4.1 and 5.8. The variation in the normal (no added zinc) rate of activation at the three pH levels is interesting.

The effects of small amounts of zinc (1 to 20 micrograms per 5 cc. substrate) on the rate of hydrolysis by crystalline trypsin and pepsin[3] was also investigated, but since the results were essentially negative (neither inhibition nor any marked stimulation) they will not be reported in detail.

Pancreatic lipase and amylase were also determined (methods of Waldschmidt-Leitz, 4). Hydrolysis of olive oil by 150 mgm. of pancreas from zinc-fed rats showed an activity of 21.9 cc. N/10 KOH in one hour at 37°C.; while the pancreas from zinc deficient rats showed an activity of 19.2 cc. N/10 KOH. In the case of the amylases the pancreases from three zinc-fed rats showed a variation in activity of 15.0 to 19.3 per cent (average 17.5 per cent) conversion of starch to glucose per 75 mgm. tissue in 30 minutes. The amylase from the pancreases of four zinc-deficient rats had an activity of 9.1 to 18.7 per cent (average 12.3 per cent) conversion.

Effect of high zinc feeding. For the sake of completeness the effect of high zinc rations on adult rats has been investigated. Feeding a ration containing 1 per cent zinc (as the sulfate) produced a marked loss of weight (40 grams in two weeks) but had no effect on the proteolytic activity of the pancreas (table 4).

Discussion. Aside from a greatly retarded growth rate no definite symptoms have been observed in the more severe zinc deficiency described in the present paper. Occasionally a slight reddening and incrustation about the eyes, nose and feet was noticed in the deficient rats, but not as severe as had previously been observed (1). This symptom, as well as the denuding (5) and greying of zinc deficient animals (2) may perhaps be explained as due to either *a*, a slower intestinal absorption, thus allowing the rat to become deficient in that nutritional factor which is relatively at the lowest level in the ration, or *b*, to an increased requirement for this

[3] We are indebted to Dr. John Northrup for supplies of these enzymes.

nutritional factor by the body cells. (The zinc deficient and added zinc rats always ate the same amounts of the vitamin supplement so the question of unequal consumption is not involved.) It would seem that the first possibility (slow absorption) is the more plausible, and this has been indicated from previous work (1). In this respect it is interesting that magnesium deficient rats are said to have an increased requirement for vitamin "B_2" (6).

The present ration is still not sufficiently low in zinc to bring about any marked reduction in the concentration of zinc in the soft tissues, although the rat does seek to maintain this apparently critical minimum concentration at the expense of growth and proliferation of new tissue. The slight differences in the concentration of zinc in the livers of the two groups can be explained as a storage factor, since the 100 micrograms of zinc per day received by the controls is somewhat in excess of their requirement. Only the bones and teeth show a marked difference in zinc concentration. This may possibly be related to the fact that bone tissue is primarily inter-cellular and the lower zinc concentration in the deficient rat bones may represent an attempt to mobilize idle zinc stores for maintenance of the essential concentration in soft, cellular tissues. The differences in the values for the small intestines probably have little significance for the washing may not have removed all intestinal contents, especially the mucous layer adhering to the walls.

As yet there is no indication what the specific function of zinc may be in the living cell. Presumably it will be found to be similar to such essential trace elements as Mg or Mn which possess a specific rôle in the activation of some enzyme or enzyme system. If such is the case it is obvious that investigation of the enzyme activity of tissues of zinc deficient animals would tell nothing, in view of the fact that the concentration of zinc in the tissues of zinc-fed and zinc-deficient rats is not significantly different with the ration used. In vitro studies have shown that a number of enzymes may be stimulated by zinc, although not specifically, other ions having the same effect. In this connection may be mentioned the work of Michaelis and Stern (7) on spleen cathepsin, Berger, Johnson and Peterson (8) on bacterial dipeptidases, the extensive work of Andreicheva (9) on a large number of hydrolytic enzymes, and the work of Neuberg (10) on dismutation of methyl glyoxal.

The lessened activity of the pancreatic enzymes of the zinc deficient rats reported in the present paper is apparently a secondary effect related to a general state of metabolic debility, which results in the less abundant formation of these enzymes. It cannot be assigned a primary rôle in the deficiency since it is not limited to a single enzyme, is not limited to the zinc deficiency, and is not restored to normal by additions of zinc, in vitro.

SUMMARY

1. The development of a new zinc low ration in which the vitamin source is a liver product extracted with dithizone to remove the zinc is described.

2. Analysis of the whole carcass and of various tissues of zinc deficient and added zinc (100 micrograms per day) rats shows, at most, only a slight decrease in the concentration of zinc in the deficient rat tissues. Bone, teeth and blood show the greatest reduction.

3. Pancreatic enzymes (especially the proteinase and amylase) show a lessened activity in the zinc deficient rats. For reasons outlined in the paper this effect has been interpreted as a secondary effect related to a general metabolic debility, and not as a primary or specific result of the deficiency.

REFERENCES

(1) Hove, E., C. A. Elvehjem and E. B. Hart. This Journal **119:** 768, 1937.
(2) Stirn, F. E., C. A. Elvehjem and E. B. Hart. J. Biol. Chem. **109:** 347, 1935.
(3) Sylvester, N. D. and E. B. Hughes. Analyst **61:** 734, 1936.
(4) Hawk and Bergeim. Practical physiological chemistry, 10th ed., 316–317, 1931.
(5) Todd, W. R., C. A. Elvehjem and E. B. Hart. This Journal **107:** 146, 1934.
(6) Watchhorn, E. and R. A. McCance. Biochem. J. **31:** 1379, 1937.
(7) Michaelis, L. and K. Stern. Biochem. Ztschr. **240:** 192, 1931.
(8) Berger, J., M. J. Johnson and W. H. Peterson. J. Biol. Chem. **124:** 395, 1938.
(9) Andreicheva, M. Bull. soc. chim. biol. **12:** 44, 1936; **16:** 1736, 1934.
(10) Neuberg, C. and K. Kobel. Biochem. Ztschr. **280:** 297, 1935.

THE EFFECT OF EXTRACT OF THE POSTERIOR HYPOPHYSIS ON THE LOSS OF WATER BY FROGS IN A DRY ENVIRONMENT[1]

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When frogs are placed in water and injected with pituitrin there occurs within 2 to 4 hours an uptake of water amounting to 15 or 20 per cent of the weight of the animals. Factors which affect this reaction have been considered in a previous communication from this laboratory (2). The mechanism of the reaction has been considered in the work of a number of investigators reviewed by Van Dyke (8) who has concluded that extract of the posterior hypophysis alters the physiology of the skin of frogs. The apparently accepted explanation is that posterior pituitary extracts alter the permeability of the skin of frogs, allowing the uptake of more than the usual amount of water which is stored in the muscles and other tissues.

The present investigation was concerned with the effect of an injection of pituitrin upon the loss of weight of frogs taken out of water. Since the loss of dry weight of frogs is negligble during the interval of a few hours used in most of the experiments, any substantial loss of weight noted may be ascribed to loss of water.

If the permeability of the skin to water be increased by pituitrin in both directions—inwards and outwards—then the injection of pituitrin into frogs out of water should produce an increased loss of water from the skin, providing the premise be correct that the sole effect of pituitrin upon the water balance of frogs be upon the skin. If pituitrin augments only the intake of water by the skin, then injections of pituitrin into frogs out of water should not influence the water content of the body, again accepting the usual explanation of the effect of pituitrin upon the water balance of frogs. The third possible result which might be obtained by injecting pituitrin into frogs out of water is that the normal loss of water from the body might be inhibited. This last was the result obtained in the experiments to be described below in which are noted the effects of various contributing factors.

If now it still be concluded that pituitrin affects the water balance of

[1] This work was aided financially by a grant from the William Spankie Memorial Endowment for Medical Research.

frogs solely through an effect upon the skin, then pituitrin must act only in one way, by inhibiting the loss of water through the skin while at the same time either not affecting or increasing the uptake of water by the skin. An increased uptake appeared more likely from the following consideration. Four hours after injecting pituitrin, a frog in water takes up 15 to 20 per cent of its weight of water; in the same interval pituitrin injected into the frog out of water but protected from air currents (vide infra) prevents a normal loss of about 1 per cent of its weight of water. During this four hour interval, pituitrin must have caused the skin to take up 15 times or more water than it would normally lose and hence, if the sole effect of pituitrin be upon the skin, it must act by inhibiting passage of water from within the skin to the surface and also by augmenting the passage of water from without the skin inwards. In only one manner could the facts be construed to fit the simpler and more acceptable hypothesis that pituitrin acts only by inhibiting loss of water from the skin. That would be so if the frog normally loses more water by the skin when it is in water than when it is out of water, since the above deductions were made from data with the frog in water in one instance and out of water in the other instance.

METHOD. The leopard frog, Rana pipiens, was used in these experiments. The general procedure was as follows. The animals were kept in water over night to assure water equilibrium. The following morning the skin was wiped dry as completely as possible, the urine expressed, the frog weighed to the nearest 0.1 gram, injected or uninjected with pituitrin as the case might be and placed in 400 cc. beakers which were covered with weighted screen tops. The frogs so arranged were assembled in groups of 6 and 3 or 4 groups constituted a day's experiment as subsequent weighings were made at hourly or shorter or longer intervals as indicated. In all experiments except where otherwise noted, the dose of pituitrin injected into the dorsal lymph sac was 0.5 international units per 10 grams body weight of Pituitrin Surgical which was supplied in generous quantities by Dr. E. A. Sharp of Parke, Davis and Company, who also donated the Pitressin and Pitocin used in this work. This dose was found by Boyd and Brown (2) to be sufficient to elicit the maximum uptake of water by frogs. It was also found, as described below, to be ample to effect the maximum inhibition of water loss. Control frogs received no injection since it was found that injecting an equivalent volume of 0.25 per cent acetic acid had no appreciable effect upon the weight of frogs. Because the loss of water by frogs varied daily with the uncontrollable factors of temperature and humidity, controls were always run simultaneously with injected animals. Experiments were performed at room temperature, except where otherwise noted, and from 6 to 60 frogs were used to compute the mean effect, depending upon the variation encountered.

RESULTS. In table 1 has been assembled a statistical summary of the effect of pituitrin upon the loss of water expressed as loss of weight of 60 skin dried frogs in an environment of air compared with that of 60 uninjected frogs used as controls. The mean initial weight of the frogs was computed and the average percentage decrease in weight each hour. In experiments where readings were not taken exactly on the hour, the hourly readings were calculated by interpolation. The standard deviation of the distribution of results averaged in each mean was then calculated, the squared differences being divided by N rather than N-1. The standard error of the mean was found by dividing the standard deviation of the

TABLE 1

A statistical summary of the loss in weight of 60 frogs out of water compared with that of 60 frogs out of water and injected with 0.5 international unit of pituitrin per 10 grams body weight

VALUE	INITIAL WEIGHT	PERCENTAGE LOSS IN WEIGHT AFTER					
		1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours
Uninjected frogs							
	grams						
Mean	22.7	1.3	2.5	3.4	4.5	5.8	6.8
Standard deviation	7.4	0.9	1.2	1.5	1.8	2.0	2.3
Standard error	0.95	0.12	0.16	0.19	0.23	0.26	0.30
Pituitrin-injected frogs							
Mean	23.1	1.4	2.2	2.6	3.1	3.7	4.7
Standard deviation	8.7	0.9	1.1	1.3	1.6	1.8	2.3
Standard error	1.1	0.12	0.14	0.17	0.21	0.23	0.30
Difference: Pituitrin-injected subtracted from uninjected							
Mean difference	+0.4	+0.1	−0.3	−0.8	−1.4	−2.1	−2.1
Standard deviation of mean difference	1.5	0.17	0.21	0.26	0.31	0.35	0.44

distribution by the square root of N. The mean difference in the percentage loss in weight hourly in injected and control frogs was determined and the standard deviation of the difference found from the square root of the sum of the squared standard errors. A mean difference was considered statistically significant when it was greater than twice the standard deviation of the mean difference (4).

Normal frogs out of water were found to lose about 1.1 per cent of their weight per hour under these conditions. During the first hour the loss was somewhat greater due probably to evaporation of some water unavoidably left on the skin when it was wiped dry after taking the animals out

of water at the beginning of the experiment. Pituitrin-injected frogs lost weight much less rapidly. During the first hour there was no difference in the loss of weight from the controls, but during the second hour the mean loss was 0.8 gram per 100 grams of frogs, during the third hour 0.4 gram, the fourth 0.5 gram, the fifth 0.6 gram and by the sixth hour the rate of loss had increased to about that in the controls, namely, 1.0 gram. The maximum inhibition of water loss occurred during the third hour during which hour the mean loss of water in the pituitrin-injected frogs was but 36 per cent of that in the controls. During the interval from the third to the sixth hours there was a statistically significant difference in the mean percentage loss in weight of the injected and control frogs.

These results demonstrate that pituitrin inhibits the loss of water of frogs taken out of water. They are of interest in demonstrating that pituitrin can affect the water balance of a normal animal out of water. Peters (7), after reviewing the literature, reached the conclusion that pituitrin has no obvious effect upon the water balance of normal animals (chiefly mammals) as measured by the output of urine. Yanagi (9) believes, from the results of previous workers and from his own experiments, that pituitrin alters the distribution of water in favor of the blood in the bodies of mammals. Burgess, Harvey and Marshall (3) found that pituitrin and pitressin had no effect upon the volume of urine produced by frogs (Rana catesbiana) after injecting water into the dorsal lymph sac. Adolph (1) reported that infundin reduced urine flow in brain-pithed frogs in an atmosphere of oxygen, obtaining under these conditions renal vasoconstriction. The effect of infundin in Adolph's experiments lasted but a few minutes and even if a transitory antidiuresis did occur in normal, intact animals (which Burgess, Harvey and Marshall appear to dispute), it could not explain the sustained water conservation noted in the present experiments. The time relations involved in the inhibition of water loss by pituitrin being similar to those in the uptake of water by pituitrin, it would appear that a similar mechanism may be involved in each case. This mechanism probably involves an altered physiology of the skin, as discussed above, possibly combined with an increased capacity of the tissues to hold water as discussed by Boyd and Brown (2).

The influence of body weight. The experiment was repeated with frogs grouped according to their body weight. The results obtained indicated that above a body weight of 15 grams all frogs responded in the same manner to pituitrin. When animals of less than 15 grams body weight were injected with pituitrin, they lost about twice as much water in 6 hours. Uninjected control frogs of less than 15 grams body weight also lost weight faster than in the higher weight animals. For example, 11 frogs averaging 11.9 grams body weight lost 8.4 per cent of their weight in 6 hours and 11 pituitrin injected frogs averaging 13.2 grams body weight lost 6.3 per cent of their weight in 6 hours. The mean difference of 2.1

per cent had a standard deviation of 0.90, indicating that pituitrin had a significant effect even on low weight frogs.

Boyd and Brown (2) found that the uptake of water by frogs injected with pituitrin was not appreciably affected by their body weight. This has been since confirmed by other workers in this laboratory with the additional information that the individual changes are more variable in low weight frogs although the means remain about the same.

Repeated injections of pituitrin. It was thought that by repeated injection of pituitrin it might be possible to completely inhibit the loss of water. Under the conditions of the experiment as outlined under the method this was not possible. In subsequent experiments it was found that the power of pituitrin to inhibit loss of water varied inversely as the intensity of the dehydrating agent. Later work showed that in these experiments the most powerful dehydrating agents were the small, imperceptible currents of air which circulated through the screen top of the beakers. When the frogs were placed in covered beakers in the ice box, it was possible to completely inhibit weight loss for several hours with an injection of pituitrin.

Since the effect of pituitrin began to wear off in 3 hours, repeated injections of the same dose (0.5 unit per 10 grams) were given at 2 hour intervals. In this manner it was possible to decrease the loss in weight to a mean of 0.6 gram/100 grams of frogs/hour as against a normal loss of 1.1 gram and a loss of 0.8 gram following a single injection of pituitrin in a period of 6 hours.

Dosage of pituitrin. The smallest dose of pituitrin which produced the maximum uptake of water by frogs was found by Boyd and Brown (2) to be 0.25 international unit per 10 grams of frog. Since the batch of pituitrin used in the present study was different from that reported before (2), it was assayed by several workers in the laboratory for its capacity to produce uptake of water. The smallest dose producing the maximum effect on water uptake was found to average 0.2 international unit. These results would suggest that the content of amphibian water balance principle of extracts of the posterior hypophysis closely follows that of the oxytocic principle against which most extracts of the posterior pituitary are standardized.

With this information, an assay was made to determine the smallest dose of pituitrin which produced the maximum inhibition of weight loss of frogs taken out of water. Frogs were assembled in groups of 6 and each group injected with one dose of pituitrin, the doses varying from 0.025 to 1.0 international unit per 10 grams body weight. Weighings were taken hourly but for the purpose of illustrating the results, the average percentage losses in weight at the end of the experiment in 6.5 hours after injection have been assembled in table 2. With doses between 0.1 and 1.0 unit, the mean loss in weight at the completion of the experiment

was 3.5 to 3.8 per cent and with doses lower than 0.1 unit per 10 grams the mean loss of weight increased. These results indicated that 0.1 international unit of the batch of pituitrin used was the smallest amount which produced the maximal inhibition of weight loss in frogs taken out of water. The smallest dose producing the maximal inhibition of water loss was thus about the same as the smallest dose producing the maximal uptake of water.

Pitressin vs. pitocin. Up to this point, two groups of data suggested that the mechanism governing the effect of pituitrin upon inhibition of water loss and stimulation of uptake of water in frogs may be identical, namely, the similar time relations and similar minimal doses producing maximal responses. Another characteristic reaction of pituitary extracts is that the pitocin fraction has a greater effect upon the uptake of water than the pitressin fraction (2). Such was not the case with the

TABLE 2

The mean loss in weight of frogs 6.5 hours after receiving injections of varying doses of pituitrin

DOSE	LOSS IN WEIGHT
Int. units per 10 grams	*Per cent of initial weight*
0.00	7.3
0.025	5.6
0.050	4.3
0.10	3.8
0.175	3.8
0.25	3.6
0.50	3.5
1.0	3.6

inhibition of water loss. A total of 246 frogs were used to establish this important differential point. In the first place, neither pitressin nor pitocin in doses up to 2 units per 10 grams body weight was capable of inhibiting water loss to the same extent as pituitrin. In the second place, individual doses of pitocin varying from 0.01 to 0.5 unit per 10 grams produced the same inhibition of water loss as corresponding doses of pitressin. For example, 6 hours after injecting 0.25 unit per 10 grams, the mean loss of weight with pituitrin was 3.6 per cent, with pitressin 4.8 per cent and with pitocin 5.1 per cent. It would appear, thus, that the pituitary control of water loss follows a similar though not identical mechanism as the pituitary control of water uptake in frogs.

The effect of light. Boyd and Brown (2) found that the uptake of water by frogs injected with pituitrin was greater when the experiment was performed in the dark room than when performed in daylight in the open laboratory and the results were taken to indicate that light had a de-

pressing effect upon the reaction. Jores (5) concluded that light may affect the output of middle and posterior lobe hormones. The whole subject of the effect of light on pituitary activity is still sub judice and is reviewed in a recent symposium, *The pituitary gland*, published by Williams & Wilkins, 1938.

As in the experiments described by Boyd and Brown (2), frogs were removed from water and assembled in screen-covered beakers in the dark room with and without illumination from an electric light in the ceiling, and in the open laboratory in and out of direct sun light. The loss of weight in the open laboratory out of direct sun light was similar to that already described. In direct sun light, the frogs lost an average of 12 per cent of their weight in 7 hours and pituitrin had but little effect upon this water loss. The effect of the dehydrating agent, in this case warm sun light, was apparently too great for pituitrin to overcome. That pituitrin cannot counteract an excessive loss of water rapidly produced in frogs was further shown by experiments in which frogs were taken out of water and placed in open wire baskets in a draft created by an electric fan. After 3 hours in such a draft, the frogs had lost 26 per cent of their weight (further loss of water killed the animals) and pituitrin injections had no effect whatsoever on the loss of water.

Experiments performed in the dark room with and without electric light were almost identical in results. The dark room was a small room about 6 feet square, without windows and without ventilation and the temperature of the dark room was kept within a fraction of a degree of that in the open laboratory by leaving the door slightly ajar but without light getting in. After 6 hours in the dark room the frogs had lost from 0.5 to 2 per cent of their weight of water compared with 6 to 7 per cent in the open laboratory. The pituitrin-injected frogs lost about half as much water as the controls. The results appeared on the surface to indicate that darkness had inhibited the loss of water of these frogs.

Alternative explanations of the effect of the dark room were sought and studied. To be brief, it was finally found that when the screen cover of the beaker containing the frog was replaced by a glass cover leaving only a small hole for air over the spout of the beaker, the loss of water in the open laboratory was also decreased to less than 2 per cent in 6 hours. With glass covered beakers the loss of water was the same in the dark room as in the open laboratory in day light and pituitrin effected the same inhibition of water loss under both light conditions. The striking difference in results with screen-covered beakers had obviously been due to the fact that there was little or no air in motion in the small, windowless dark room and considerable, though not evident, air in motion in the open laboratory even after the screen-covered beakers had been purposely assembled away from obvious drafts. It may thus be concluded that darkness does not inhibit the loss of water by frogs and that light does not

increase the loss of water except where it raises the temperature which is the probable explanation of the results in sun light.

The above observations may serve to explain the unusual results of Oldham (6) who assembled frogs in wire cages, the bases of which were placed 1 cm. below the surface of water in a large, open water bath. When pituitary extract was injected into such animals, a maximum gain in weight of only 4 per cent was obtained as against the usual increase of 15 to 20 per cent when frogs are assembled almost completely immersed in water (2). Apparently in Oldham's experiments air in motion produced a considerable loss in weight by evaporation of water from the skin and thereby prevented a maximal increase in weight when pituitrin was injected.

Refrigerator room experiment. When frogs were assembled in beakers in the refrigerator room at a temperature of 4°C., the normal loss of water was only about $\frac{1}{6}$ to $\frac{1}{7}$ that at room temperature. Pituitrin injected into these animals completely inhibited the loss of water for a period of 4 to 6 hours, after which loss of water proceeded at the same rate in injected and control animals.

SUMMARY

The loss of body water by leopard frogs taken out of water is inhibited by pituitrin providing that evaporation from the skin is not excessive such as occurs in warm sun light or in a forced draft from a fan. Imperceptible air currents markedly affect evaporation of water from the skin of frogs, the rate of water loss in a wire covered beaker in a ventilated room being 6 times that in glass covered beakers. The loss of water is not increased in the dark but is much less at low temperatures. Very small frogs lose relatively more water than frogs of normal size. The smallest dose of pituitrin which produces the maximum inhibition of water loss is about the same as that which produces the maximum uptake of water by frogs. The length of time over which pituitrin is effective is also about the same in frogs out of water as in frogs in water. Pitressin and pitocin are equally effective in inhibiting loss of water and neither one as effective as pituitrin in this respect.

REFERENCES

(1) Adolph, E. F. This Journal **117**: 366, 1936.
(2) Boyd, E. M. and G. M. Brown. This Journal **122**: 191, 1938.
(3) Burgess, W. W., A. M. Harvey and E. K. Marshall. J. Pharmacol. and Exper. Therap. **49**: 237, 1933.
(4) Davenport, E. B. and M. P. Ekas. Statistical methods in biology, medicine and psychology. 4th ed., Wiley, New York, 1936.
(5) Jores, A. Klin. Wchnschr. **14**: 1713, 1935.
(6) Oldham, F. K. This Journal **115**: 275, 1936.
(7) Peters, J. P. Body water. Thomas, Baltimore, 1935.
(8) Van Dyke, H. B. The physiology and pharmacology of the pituitary body. Univ. of Chicago Press, 1936.
(9) Yanagi, K. J. Pharmacol. and Exper. Therap. **56**: 23, 1936.

RÔLE OF PROGESTERONE AND OTHER HORMONES IN SURVIVAL OF PSEUDOPREGNANT ADRENALECTOMIZED FERRETS[1]

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Dogs adrenalectomized while in estrus fail to develop adrenal insufficiency during the subsequent pseudopregnant period (1, 2). The authors found the same was true for ferrets (preliminary reports, 3, 4), and similar phenomena have since been found in cats (5). The same results to a less marked extent follow pituitary therapy in rats (6, 7). Thorn and Harrop (8) and Thorn and Engel (9) found similarities (and some differences) in the effect of sex and cortical hormones on electrolyte excretion of intact dogs, and recently other work has appeared showing the fluid-retaining effects of sex hormones (Kenyon *et al.* and others, 10).

No satisfactory analysis, however, has been made of factors permitting pseudopregnant animals to live after adrenalectomy. Despite their effect on electrolyte metabolism, estrogenic hormones are not only useless but toxic to adrenalectomized animals (7, 11, 12, 13). Androgens likewise are not beneficial (13, 14, 9). Progesterone, the most promising substance, in view of the effects of pseudopregnancy, has, in tests in at least five laboratories, hitherto failed to substitute for the cortical hormone in adrenalectomized animals (2, 11, 7, 13, 15). A related synthetic substance, desoxy-corticosterone of Steiger and Reichstein, was, however, effective (15, 16).

In the ferret, because of the nature of its reproductive cycle, we found it possible to analyze the separate rôles of estrus and pseudopregnancy in adrenal insufficiency more easily than in forms previously used, and in the course of this study the probable life-maintaining factor of pseudopregnancy was identified.

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The authors are indebted to Prof. W. W. Swingle who furnished all of the cortical extracts used here and provided other assistance.

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MATERIALS AND METHODS.[2] Ferrets 6 to 9 months old were obtained from various dealers. They were fed the diet most appetizing following adrenalectomy: fresh ground, lean meat (to which was added, 1 per cent Na-chloride, 0.5 per cent Na-bicarbonate, and 0.5 per cent Na-citrate) and fresh milk. They were kept singly in large cages at about 77°F. Adrenalectomy, under nembutal anesthesia, was done in two stages. Cortical extract was generally given after operation until wounds were healed and appetites normal. After extract withdrawal a state termed "pre-terminal" adrenal insufficiency, characterized by asthenia, weight loss, and refusal of food for at least 24 hours, appeared in an average of 15 cases in 6 days. At this stage recovery measures were instituted. If untreated, death invariably occurred in 1 to 3 days. Revival from adrenal insufficiency was accomplished by injections of 4 to 5 cc. cortical extract per day in divided doses of 1 cc. each, together with oral administration (by pipette) of 15 per cent glucose solution to which the Na-salt mixture used in the meat was added to the limit of tolerance. When back in good health 1 cc. of cortical extract per day was, uniformly, an adequate and practically minimal, maintenance dose.

Response to spontaneous estrus, gonadotropic agents and estrogenic hormone. From the work of Rogoff and Stewart on the dog many have referred to the ameliorating effect of estrus on adrenal insufficiency, but estrus is a transient period in the dog followed by a spontaneous pseudopregnancy. A clean-cut distinction can be made in the ferret between the effects of estrus and pseudopregnancy, because these animals are non-spontaneously ovulating and remain in estrus throughout the breeding season unless mated. Adrenalectomized ferrets on maintenance doses of cortical extract came into estrus normally. One to 2 weeks after estrus appeared, however, a severe adrenal insufficiency, demanding vigorous resuscitation measures, invariably appeared unless the cortical hormone doses were at least doubled. Likewise when estrus was induced by the gonadotropic agents described below, it was necessary to increase the dose of cortical extract from 2 to 4 times.

When 2,000 I.U. Amniotin daily were given to an adrenalectomized, ovariectomized female, appetite failed in 24 hours and death occurred in four days. One thousand I.U. daily of Amniotin were given to an ovariectomized and incompletely adrenalectomized female, which had

[2] This work was made possible by generous supplies of hormones given us as follows: progesterone (Proluton) and testosterone (Oreton), by Dr. Erwin Schwenk of the Schering Corporation; Amniotin, Follutein, and anterior pituitary extract, by Dr. J. A. Morrell of E. R. Squibb & Sons; pregnancy mare serum (Gonadin) by Mr. Donald Wonder of the Cutter Laboratories. Dr. George W. Thorn of Johns Hopkins Hospital helped supply progesterone for an initial exploratory experiment.

shown signs of chronic adrenal insufficiency but nevertheless had lived for four months without any treatment. Her appetite failed on the 4th day of treatment and death occurred on the 14th.

It seems clear, therefore, that the secretion of estrogen, even in normal physiological amounts, or its administration in greater amounts, precipitates rather than prevents adrenal insufficiency.

Effect of natural and induced pseudopregnancy. Pseudopregnancy, which lasts about 6 weeks (17), was produced during the breeding season by breeding estrous animals to vasectomized males. Five adrenalectomized ferrets, made pseudopregnant, remained in excellent health for 27 to 37 days when all therapeutic measures, including the salt supplement of the diet, were withdrawn (table 1). Cortical extract could not, however, be withdrawn with safety until about 3 days after ovulation.

During the winter anestrous season pseudopregnancy was induced by menopause urine extracts (Gamone—Squibb) or pregnant mare serum

TABLE 1

Survival of untreated, pseudopregnant, adrenalectomized ferrets

NUMBER FERRETS USED	PSEUDOPREGNANCY-PRODUCING STIMULUS	AVERAGE DAYS LIVED WITHOUT CORTICAL EXTRACT AND RANGE
5	Sterile mating	31 (25–37)
7	Gonadotropic hormones	32 (15–46)
Average of untreated, anestrous and male ferrets (15 cases).		6 (3–10 .5)

(Gonadin—Cutter) used either alone or followed by pregnancy urine prolan (Follutein—Squibb). Attempts were made to give dosages which would induce initial estrous changes, as judged by vulval response, vaginal smears and mating behavior, and lead to eventual luteinization, i.e., pseudopregnancy. In seven animals in which pseudopregnancy was thus apparently induced, cortical hormone was withdrawn and no symptoms of adrenal insufficiency appeared for an average of 31 days (table 1).

Removal of the ovaries from two untreated, pseudopregnant, adrenalectomized ferrets resulted in the appearance of "pre-terminal" adrenal insufficiency in 4 days. The luteinized ovary is therefore essential for this life-extending effect.

Effect of crystalline progesterone and corpus luteum extracts. Above results indicated either that progesterone or some unknown cortical-like secretion of the corpus luteum must be the factor mitigating adrenal insufficiency during pseudopregnancy. Despite previous failures in other forms we re-investigated the effects of crystalline progesterone, which

was kindly supplied us in sizable amounts by Dr. Erwin Schwenk. A preliminary note on these results has been published (18).

Three anestrous females were treated for 14, 30 and 33 days; two castrate females for 20 and 32 days; one castrate male for 25 days; and one female for 3 days in a revival experiment. With dosages of 2 to 5 mgm. per day all animals were maintained in the best of condition, gaining weight steadily, throughout the duration of treatment. Apparently life could have been maintained indefinitely. In an attempt to arrive at a minimal effective level, the dose in 3 animals was gradually reduced from 5 to

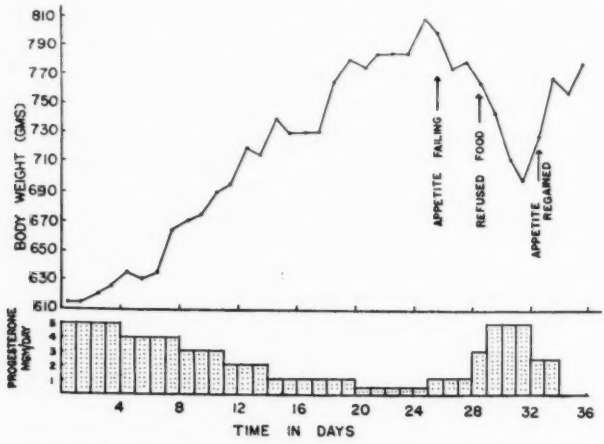

Fig. 1. Body weight curve of an adrenalectomized ferret (no. 13) given varying dosages of progesterone. Note the increasing body weight, despite decreasing dosage, until a dose of 0.5 mgm. was reached. A slow absorption of the large initial doses probably accounted for the apparent maintenance at the 1 mgm. level. A rather severe adrenal insufficiency developed by the twenty-eighth day which was relieved by 5 mgm. progesterone per day. The rapid growth occurring during progesterone treatment was such that subsequent adrenal insufficiency did not cause a return even to the original body weight levels of good health.

0.5 mgm. per day. The latter was inadequate and in all 3 cases appetite failed and adrenal insufficiency was obvious (fig. 1). These animals were, however, completely resuscitated by the use of 5 mgm. progesterone per day, as was a fourth animal revived in the course of another experiment. Although enough figures are not available for a definite statement, our impression is that the minimal effective dose is about 2 mgm. or less per day.

If this estimate is correct, it appears on the basis of known minimal needs for corticosterone and cortical extract in the dog, and of cortical

extract in the ferret, that progesterone is in the range of one-third as effective as corticosterone.

A commercial corpus luteum extract, in doses of 2 I.U. per day was toxic, perhaps because of estrin contamination. It caused loss of appetite, etc., within 24 hours in two cases.

As in other species, the occurrence of spontaneous estrus was prevented in all cases by the chronic administration of progesterone.

Effect of testosterone propionate. Effects of testosterone propionate were observed in 5 adrenalectomized female ferrets, 3 of which were castrates. On doses of 3 (1 case), 5 (1 case) and 10 mgm. per day, all animals died within 8 to 13 days. While non-beneficial, this substance was, unlike estrin, non-toxic.

Effect of anterior pituitary extract. Anterior pituitary extract (Squibb) was found by Swingle *et al.* (19) to extend the lives of adrenalectomized cats, males, females or castrates. The material contains gonadotropic elements— it increased ovarian weights in immature rats, produced ovulation in estrous ferrets, and induced intense interstitial tissue development in ovaries of anestrous ferrets thus preventing estrus. Its ability, however, to maintain castrate cats indicated that its effective action was not entirely gonadotropic.

Five adrenalectomized female ferrets (2 castrated) were given this extract in doses of 1 or 2 cc. per day. "Pre-terminal" adrenal insufficiency appeared in 3, 5, 5, 6, and 13 days, indicating, as in rats (7), that the material was non-beneficial. A peculiar species difference is indicated, for of the forms studied, the cat alone has been benefited by this extract.

Discussion. The old enigma of the extended life-span of pseudopregnant, adrenalectomized animals seems adequately explained by the secretion of progesterone alone. No adequate explanation is available, however, for the adverse effects of estrus and the estrogens after adrenalectomy. The suggestion that estrin is toxic because it inhibits the pituitary (12, 13) has not been substantiated by experiments in which estrin plus pituitary extract was given to adrenalectomized rats (Gaunt and Edelman, unpublished). The toxicity of estrin was not thereby reduced.

Steiger and Reichstein (15) have suggested, in view of their belief that progesterone had no cortical hormone-like activity, that the 21-oxy group of the adrenal hormone molecules was essential for their characteristic action. Our results indicate either that this view should be modified or that the tissues of an adrenalectomized (and gonadectomized) animal of either sex can convert progesterone into a 21-oxy form.

The cortical hormone-like action of progesterone is apparent at a dosage level of physiological significance. This leads to a consideration of the possibility that the function of progesterone in preparation for the rigors

of pregnancy may be more generalized than its action on the reproductive tract alone. Cole and Hart (20) have shown for instance that pseudopregnancy, as well as pregnancy, increases appetite and growth, and the appetite and weight increases in our progesterone-treated ferrets resembled the phenomena described by them (see fig. 1).

SUMMARY

In a study of adrenal-gonad interrelationships as observed in adrenalectomized ferrets, the following facts were established: 1. Spontaneous estrus, estrus induced by gonadotropic agents or estrin, all accelerate the appearance of adrenal insufficiency, and the cortical hormone requirements of an animal in estrus are at least doubled. 2. The condition of pseudopregnancy, contrariwise, induced either by sterile mating following spontaneous estrus, or by gonadotropic agents, prevents adrenal insufficiency; and adrenalectomized pseudopregnant ferrets require no cortical hormone. This prevention of adrenal insufficiency is dependent upon the presence of luteinized ovaries. 3. The absence of adrenal insufficiency during pseudopregnancy is probably due to the secretion of progesterone, inasmuch as crystalline progesterone in minimal doses approximating 2 mgm. per day will maintain adrenalectomized ferrets, either males, females or castrates, in excellent health without any additional treatment. In larger doses (5 mgm. per day) it will relieve an established adrenal insufficiency.[3] 4. Testosterone propionate is non-toxic and non-beneficial to adrenalectomized ferrets. 5. An anterior pituitary extract, effective in maintaining the lives of castrate, adrenalectomized cats was non-beneficial in ferrets. 6. Spontaneous estrus is prevented in the ferret by the chronic administration of progesterone.

REFERENCES

(1) Rogoff, J. M. and G. N. Stewart. This Journal **86:** 20, 1928.
(2) Swingle, W. W., W. M. Parkins, A. R. Taylor, H. W. Hays and J. A. Morrell. This Journal **119:** 675, 1937.
(3) Gaunt, R. Cold Spring Harbor Symp. Quant. Biol. **5:** 395, 1937.
(4) Gaunt, R. and H. W. Hays. Anat. Rec. (Suppl.) **70:** 29, 1938.
(5) Collings, W. D. Thesis, Princeton Univ. Library, 1938.
(6) Emery, F. E. and E. L. Schwabe. Endocrinology **20:** 550, 1936.
(7) Cavanaugh, C. J. and R. Gaunt. Proc. Soc. Exper. Biol. and Med. **37:** 226, 1937.
(8) Thorn, G. W. and G. A. Harrop. Science **86:** 40, 1937.
(9) Thorn, G. W. and L. L. Engel. J. Exper. Med. **68:** 299, 1938.
(10) Kenyon, A. T., I. Sandiford, A. H. Bryan, K. Knowlton and F. C. Koch. Endocrinology **23:** 135, 1938.

[3] Gaunt, Nelson and Loomis (in press) have found that progesterone will likewise maintain adrenalectomized rats, but the dosage necessary, on the basis of equivalent body weight is 10 or more times that needed for the ferret.

(11) SCHACHER, J., J. S. L. BROWNE AND H. SELYE. Proc. Soc. Exper. Biol. and Med. **36:** 488, 1937.
(12) GAUNT, R., H. E. POTTS AND E. LOOMIS. Endocrinology **23:** 216, 1938.
(13) D'AMOUR, F. E. AND D. FUNK. J. Pharmacol. and Exper. Therap. **62:** 307, 1938.
(14) SPURR, C. L. AND C. D. KOCHAKIAN. Proc. Am. Physiol. Soc., p. 193, 1938.
(15) STEIGER, M. AND T. REICHSTEIN. Helv. Chim. Acta **20:** 1164, 1937.
(16) SIMPSON, S. L. Lancet **2:** 557, 1938.
(17) HAMMOND, J. AND G. H. A. MARSHALL. Proc. Roy. Soc., B **105:** 607, 1929.
(18) GAUNT, R. AND H. W. HAYS. Science, in press.
(19) SWINGLE, W. W., W. M. PARKINS, A. R. TAYLOR, H. W. HAYS AND J. A. MORRELL. This Journal, in press.
(20) COLE, H. H. AND G. H. HART. This Journal **123:** 589, 1938.

THE ACTION OF INSULIN AND OF ANTERIOR PITUITARY EXTRACT IN NORMAL AND HYPOPHYSECTOMIZED RATS[1]

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Although the phenomenon of hypersensitivity to insulin after hypophysectomy was the first experimental finding to relate the hypophysis to carbohydrate metabolism, the reason for this hypersensitivity is obscure. Likewise, the mechanism by which anterior lobe extracts counteract the effects of insulin in normal and hypophysectomized animals is unsettled. The literature concerning this subject has been reviewed recently (1, 2). Several possible explanations have been considered, but, as discussed in a later section of this paper, the evidence for none of these views can so far be deemed conclusive.

In recent work it has been found that when hypophysectomized rats are fed glucose, they deposit much less of the absorbed carbohydrate as glycogen and apparently oxidize more of it than do normal rats (3). Extracts of the anterior pituitary were found to depress the respiratory quotients and apparent carbohydrate utilization of both hypophysectomized and normal rats and to promote the preservation of the carbohydrate as glycogen and tissue glucose. In view of these results, it seemed advisable to study the effects of insulin, and of pituitary extracts administered with insulin, on carbohydrate levels and respiration under conditions similar to those employed in the previous experiments (3).

METHODS. In all of the experiments reported here young male rats, weighing 160 to 180 grams when fasted, were used. Hypophysectomies were performed when the rats were 50 to 60 days of age, and the animals used for these experiments 3 to 5 weeks later. The completeness of the removal of the gland was in all cases checked by inspection at autopsy and by records of growth and of gonadal and adrenal atrophy.

The rats were fasted under uniform conditions, the normal rats 24 hours

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and the hypophysectomized animals 18 hours, before all experiments were begun. Injections of A.P. extracts were made intraperitoneally, either at intervals during the fasting periods and also one hour preceding the experiment, or once only, one to two hours before the experiment. At least one hour after the last injection of A.P. extract, glucose was fed in constant volumes of 25 and 40 per cent solutions to hypophysectomized and to normal rats respectively. A stomach tube was used without anesthesia. At the same time that the glucose was fed, or in the fasted series, one hour after the last injection of A.P. extract, insulin (Lilly) was injected intraperitoneally in a volume of not more than 0.3 ccm.

Following the feeding and insulin injection, the rats were placed in a Kleiber respiration apparatus, described previously (3), and the average respiratory quotients determined over the next 3 hours, after time taken for equilibrium of the apparatus. The temperature in the respiratory chamber was maintained at 27°C. during the experiments. All metabolism measurements were made between the hours of 10 a.m. and 2 p.m. After this period, the animals were anesthetized with sodium amytal, and 4 hours after glucose was fed or insulin injected, samples of blood and of muscle (gastrocnemius) and the whole livers were taken for analysis. From the fed animals, the whole gastro-intestinal tracts were removed, placed in warm water, slit lengthwise and extracted several times with hot water. All but negligible amounts of non-fermentable reducing material were removed from these extracts by precipitation with the Somogyi $ZnSO_4$ reagent (10 ccm. of 10 per cent $ZnSO_4 \cdot 7H_2O$ and 10 ccm. 0.5 N NaOH). Analyses were also made of aliquot samples from all of the glucose solutions which were fed. A modification of the Good-Kramer-Somogyi technique for glycogen determinations was used; the Somogyi $CuWO_4$ reagent was employed as blood precipitant and the Shaffer-Somogyi Reagent 50 used for all glucose determinations.

The anterior pituitary extract used in these experiments was made from dissected beef glands by a procedure standard in this laboratory (5); samples from one batch only (B 479) were used. It contained large amounts of the "glycostatic" factor, as well as growth, thyrotropic, and adrenotropic hormones, and some lactogenic and gonadotropic factors. Each ccm. contained 14 mgm. of organic material. The total dose given was 1 ccm. when one injection only was made, and 1.5 ccm. when injections were made during the fasting period. This dosage is 8 and 12 times the minimum effective dose for maintenance of muscle glycogen levels in fasted hypophysectomized rats, and 4 or 6 times the amount necessary for a maximal effect (6).

Results. The results of the investigations reported here are presented in the accompanying tables, and discussed below. The figures are in most cases averages of closely agreeing series; to save space, the probable errors

of these averages have been included only where most important. Since several of the control series appear in more than one table, probable errors have usually been given only in the first table, to which reference is made. All figures representing the results of glycogen determination have been expressed as glucose.

1. *The action of insulin in fasted rats.* A comparison of the effects of graded doses of insulin in normal and in hypophysectomized fasted rats is presented in table 1. Here the enormous hypersensitivity to insulin of the operated rats is again demonstrated; for in these animals 0.1 unit

TABLE 1

The action of insulin on fasted hypophysectomized and normal rats

Respiratory data obtained during 4 hours after injection of insulin. Terminal analyses at 4¼ hours

	NUMBER OF RATS	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q.
		mgm. per cent	*mgm. per 100 grams body wt.*	*mgm. per cent*	*ccm. per 100 gm. per hour*	
A. Hypophysectomized rats						
1. No insulin	12	58 ±3.1	0.5	350	88 ±1.3	0.787 ±0.009
2. Insulin, 0.1 unit per kilo	7	16 ±1.9	0.5	170	68 ±1.3	0.854 ±0.010
3. Adrenal-demedullated, insulin, 0.05 unit per kilo	7	34 ±4.8	0.4	409	61 ±3.3	0.800 ±0.008
B. Normal rats						
1. No insulin	15	72 ±2.0	1.2	524	154 ±1.1	0.724 ±0.003
2. Insulin, 0.1 unit per kilo	6	77 ±4.3	0.9	510	141 ±2.1	0.737 ±0.005
3. Insulin, 1.0 unit per kilo	9	54 ±3.5	3.5	393	138 ±2.5	0.767 ±0.005
4. Insulin, 3.0 units per kilo	4	26 (14–46)	3.3	417	141	0.748

of insulin per kilo produced about the same relative change in blood sugar level as that brought about by 3.0 units per kilo in the normal rats—a thirty-fold difference.

A remarkable decrease in the oxygen consumption of the insulinized hypophysectomized rats is the most noteworthy feature of these experiments. One-tenth unit per kilo of insulin produced a decrease of about one-third in a metabolic rate already only 60 per cent of the normal; whereas but a small and barely significant fall in oxygen consumption was produced by the largest doses of insulin used in the normal rats (3 units per kilo).

As pointed out later, this fall in oxygen consumption does not seem to be explicable as a pre-mortal effect of the very low blood sugar levels produced at the same time.

Along with the decrease in oxygen consumption in the hypophysectomized rats given insulin, there occurred a marked rise in the R.Q. which was not observed in the normal rats. This change would seem to indicate that these animals were also abnormally sensitive to the action of insulin in increasing oxidation of carbohydrate. However, in these experiments the terminal blood sugar levels were very low and there had evidently been much glycogenolysis in the muscle tissue; so that there could well have been acidosis sufficient to affect the observed R.Q.'s. The same consideration applies to the normal series in which larger doses of insulin were given. Further, the decrease in muscle glycogen was shown to be an indirect effect by the fact that in demedullated animals, either hypophy-

TABLE 2

The action of insulin in fasted adrenal-demedullated and hypophysectomized rats

Determinations made before and 2 hours after injection of insulin, with amytal anesthesia in interim. Groups A and B fasted 24 and 18 hours respectively.

	NUMBER OF RATS	BLOOD SUGAR	MUSCLE GLYCOGEN
		mgm. per cent	*mgm. per cent*
A. Adrenal-demedullated			
1. Before insulin	4	68	522
2. After 0.05 u. insulin per kilo	4	49	530
B. Hypophysectomized, adrenal-demedullated			
1. Before insulin	5	39	419
2. After 0.05 u. insulin per kilo	5	16	374

sectomized or otherwise normal, no great change in the muscle glycogen values was produced when insulin was administered (tables 1 and 2). Because of the great hypersensitivity to insulin of the doubly operated rats, only 0.05 unit per kilo of insulin could be given to them. In this series the average R.Q. was not significantly greater than in untreated fasted hypophysectomized rats, although still far above that for normal fasted animals.

These results of insulin administration to fasted rats, because of the complications brought on by the low blood sugar levels, do not permit conclusions as to the mechanism of insulin action. It therefore became necessary to study the effects of insulin in animals which had been fed carbohydrate.

2. *The action of insulin in fed rats.* Hypophysectomized and normal rats were fasted and then fed known amounts of glucose, and graded doses

of insulin were administered at the time the glucose was fed. The effects of insulin on the carbohydrate levels and respiration are presented in table 3. In all experiments of this type, the amount of absorption of the fed glucose was determined; and in any case in which the absorption rate

TABLE 3

The effect of insulin on the metabolism of fed glucose

Insulin given intra-peritoneally immediately after feeding of glucose; terminal analyses made approximately 4½ hours after feeding

	NUMBER OF RATS	GLUCOSE ABSORBED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q.	NITROGEN EXCRETION	R.Q. NON-PROTEIN	CARBOHYDRATE OXIDIZED		PER CENT OF TOTAL ENERGY FROM CARBOHYDRATE
										Mg. per 100 gm.	Per cent of amount absorbed	
		mg. per 100 gm. per hour	*mg. per cent*	*mg. per 100 gram body wt.*	*mg. per cent*	*cc. per 100 gm. per hour*		*mg. per 100 gm. per hour*				
A. Normal rats												
1. No insulin	10	189	132	123	762 ±13	147 ±2.1	0.857 ±0.005	1.9	0.862	453	53 ±1.5	55
2. 0.1 unit per kilo	4	192	132	99	768	153	0.842	4.0	0.850	362	45	51
3. 1.0 unit per kilo	7	199	125	103	863 ±18	145 ±2.3	0.853 ±0.017	2.9	0.861	387	46	54
4. 2.5-5.0 units per kilo	5	192	82	36	857	151						
5. 10.0 units per kilo	8	201	82	84	1010 ±25	150 ±1.6	0.887 ±0.007	2.2	0.896	508	59	66
B. Hypophysectomized rats												
1. No insulin	10	122	79	25	520 ±10	107 ±2.7	0.908 ±0.007	2.2	0.925	420	74 ±3.7	76
2. 0.1 unit per kilo	10	126	82	31	495	100 ±2.5	0.933 ±0.009	1.5	0.947	445	80 ±3.8	83
3. 1.0 unit per kilo	10	130	64	17	504	90 ±2.6	0.951 ±0.004	1.9	0.968	401	73	90
4. 2.2-5.0 units per kilo	7	128	55	10	522	83 ±4.3						
5. 10.0 units per kilo (including 3 rats also demedullated)	7	133	41	1.6	528	75 ±3.9	0.975 ±0.007	1.0	0.986	375	72	96

was obviously abnormal for the condition studied, the experiment was discarded. In normal rats, as expected, the primary effect of insulin seems to have been the promotion of carbohydrate deposition as muscle glycogen. The deposition of liver glycogen was diminished, and when larger doses of insulin were given, the blood sugar levels were lower than

in untreated animals. The oxygen consumption was unchanged, and only when the largest dose of insulin was given (10 units per kilo) was a barely significant increase in R.Q. obtained.

The hypophysectomized rats were not as hypersensitive to the hypoglycemic action of insulin as they had been when fasted, although blood sugar and liver glycogen levels were diminished when the larger doses of insulin were given. No increases in muscle glycogen were obtained. *There was a progressive fall in oxygen consumption with increasing doses of insulin, although in no cases were blood sugar levels reduced critically.* The respiratory quotients were increased in all series, and this occurred also in adrenal demedullated, hypophysectomized rats. At first it would appear that there had been a considerable increase in carbohydrate oxidation in these animals. However, calculation of the oxidation rate indicates that it remained virtually unchanged, although the percentage of total energy apparently being obtained from carbohydrate was greatly increased. Calculations from such experiments as these cannot be precise; and inasmuch as there was greater disappearance of carbohydrate in the operated animals given insulin than in any of the other series, there may have been some increase in the oxidation of carbohydrate in this case. However, this effect cannot have been very marked.

3. *The effects of anterior lobe extracts on the action of insulin in fasted rats.* It has not been possible heretofore to demonstrate any action of A.P.E. in normal fasted rats, otherwise untreated. However, the action of A.P. preparations in preventing insulin hypoglycemia has been described by several investigators. Therefore, the combined action of A.P.E. and insulin on the glycogen levels and the respiration of normal fasted rats was determined (table 4). When the A.P. extract was given during the preceding fasting period, all trace of the action of 1 unit of insulin per kilo on carbohydrate levels and on respiration was prevented completely. However, when the dose of A.P. was given but one hour before the insulin, the terminal blood sugar levels were not restored, although the fall may have been slower, as shown by the small amount of glycogenolysis in the muscle tissues. Even in this case, the extract prevented entirely any fall in oxygen consumption and also prevented the small rise in R.Q.

Anterior pituitary extracts when administered to fasted hypophysectomized rats prevent the usual fall in muscle glycogen levels and restore the R.Q.'s to normal, but they do not noticeably affect the blood sugar levels. When 0.1 unit of insulin per kilo was given to hypophysectomized rats which had been injected during the fasting period with the usual dose of A.P. extract, the hypoglycemic action of the insulin was prevented, but the spontaneous fall in blood sugar level was not affected. With the usual amount of A.P.E., the insulin still produced a fall in oxygen consumption; but double the dose of the A.P.E. was sufficient not only to

prevent any decrease, but to raise the oxygen intake above the untreated level. The average R.Q.'s were a little above but probably not significantly different from those in the treated animals without insulin. Evidently then, in both normal and hypophysectomized fasted rats, A.P.E. may not only prevent the hypoglycemic action of insulin, but it also prevents any effect of the insulin on the metabolic rates or the respiratory quotients. However, the amount of A.P.E. required to counteract a given dose of insulin is very much greater in hypophysectomized than in normal rats.

4. *The effects of anterior lobe extract on the action of insulin in fed rats.* In most respects, insulin and A.P.E. have been found to have opposite effects. However, in one case the end effects of the two substances are

TABLE 4

The effects of insulin and of A.P. extracts on normal fasted rats

Respiratory data obtained during 4 hours after injection of insulin. Terminal analyses at 4½ hours

	NUMBER OF RATS	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q.
		mgm. per cent	*mgm. per 100 grams body wt.*	*mgm. per cent*	*ccm. per 100 grams per hour*	
1. Untreated rats (from table 1)	15	72	1.2	524	154	0.724
2. Insulin, 1 unit per kilo, (from table 1)	9	54	3.5	393	138	0.767
3. Insulin, 1 unit per kilo, A.P.E.*	6	58	3.3	485	150	0.738
4. Insulin, 1 unit per kilo, A.P.E. during fast†	5	79	6.0	549	150	0.729

* One cubic centimeter standard alkaline extract given 1 hour before insulin injection.

† Standard alkaline extract given in 3 doses of 0.5 ccm. each, at 24 and 18 hours and at 1 hour before insulin injection.

the same: both cause a marked increase in the amounts of glycogen deposited in the muscles of fed normal rats. This effect of insulin has been known for some years, and that of the A.P.E. has been demonstrated recently (3, 7). Now, when both insulin and A.P.E. were given to fed rats, it was found that the effect on glycogen deposition was not additive, but was in fact less than when the insulin was given alone (table 6). Evidently, the two substances produce their effects by different and in some sense opposing physiological paths.

The A.P.E. also caused a drop in the average R.Q. of the normal rats whether insulin was given or not, and quite prevented the fall in blood sugar level when 10 units per kilo of insulin were given. When 20 units per kilo were given with the same amount of A.P.E., there was still de-

pression of the R.Q.'s, and only a small drop in blood sugar level; the muscle glycogen deposition, however, was marked. The changes in liver glycogen occurring in these series do not easily admit of interpretation because of their variability and because of alterations in the absorption rates in injected animals which may have affected the liver glycogen values. Although two series were run in these experiments, one in which the A.P.E. was given only just before the experiment and the other in which it had been given during the preceding fast or longer, no differences in the results were detected. The figures for the two series were therefore combined into one set of averages in each case, as presented in table 6. We may conclude that in normal fed animals, A.P.E. in the presence of large amounts of insulin continues to demonstrate its depressing activity on carbohydrate

TABLE 5

The action of insulin and A.P. extract on fasted hypophysectomized rats

Terminal analyses made 4 hours after insulin injection. Respiratory data obtained over 3 hour period in interim

	NUMBER OF RATS	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q.
		mgm. per cent	*mgm. per 100 grams body wt.*	*mgm. per cent*	*ccm. per 100 grams per hour*	
1. Untreated rats (from table 1)	10	58	0.5	350	88	0.787
2. Insulin, 0.1 unit per kilo (from table 1)	7	16	0.5	170	58	0.854
3. A.P.E.*	5	48	0.4	565	93	0.736
4. Insulin, 0.1 unit per kilo plus A.P.E.						
a. 1.5 ccm. A.P.E.*	5	44	0.4	460	76	0.751
b. 3.0 ccm. A.P.E.*	5	52	0.6	567	101	0.752

* Standard alkaline extract given in 3 doses of 0.5 or 1 ccm. each, at 24 and 18 hours and at 1 hour before insulin injection.

utilization, and at the same time prevents in whole or in part the usual increase in the formation of muscle glycogen expected after the insulin administration.

The effect of A.P.E. on insulin action in hypophysectomized rats was next investigated. As shown in table 7, it was found that the usual dose of A.P.E. given one hour before the glucose feeding prevented completely the effects of 1 unit but not of 10 units of insulin per kilo on oxygen consumption, R.Q.'s and blood sugar levels. Liver glycogen formation was still low, probably because of the somewhat lower absorption rates prevailing in the injected hypophysectomized rats.

In these experiments, the injections of A.P.E. were first given only just before the glucose was fed, since otherwise the muscle glycogen levels would

have been altered and a comparison of the carbohydrate levels after feeding with those found in untreated fed rats would have become impossible. In another series, therefore, A.P.E. was given alone during the fasting period preceding the experiment, raising the muscle glycogen levels to normal without affecting liver glycogen or blood sugar values (table 5). When glucose was fed to these animals without further treatment, there was almost normal deposition of muscle glycogen and some increase in liver glycogen over that found in untreated fed rats (table 7). The further addition of 10 units per kilo of insulin to this A.P. treatment was then almost completely counteracted. Considering the difference in absorption

TABLE 6

*The effects of insulin and of A.P.E. on the metabolism of normal rats fed glucose**

	NUMBER OF RATS	GLUCOSE ABSORBED	BLOOD GLUCOSE	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q. UNCORRECTED
		mgm. per 100 grams body wt.	*mgm. per cent*	*mgm. per 100 grams body wt.*	*mgm. per cent*	*ccm. per 100 grams per hour*	
1. Without treatment (from table 3)	10	854	132	123 ±4	762 ±13	147 ±2.1	0.857 ±0.005
2. Insulin, 10 units per kilo (from table 3)	8	859	82	84 ±6	1010 ±25	150 ±1.6	0.887 ±0.007
3. A.P.E.†	13	765	143	115 ±6	871 ±17	166 ±1.9	0.781 ±0.006
4. Insulin, 10 units per kilo, plus A.P.E.‡	9	735	139	89 ±8	872 ±15	161 ±2.2	0.787 ±0.004
5. Insulin, 20 units per kilo, plus A.P.E.§	5	764	115	81	1116	160	0.779

* Insulin injected at time glucose was fed. Terminal analyses made approximately 4½ hours after feeding. All animals fasted 24 hours before feeding.

† One cubic centimeter standard alkaline extract 1 to 2 hours before feeding; or 1 ccm. standard alkaline extract per day for 3 days, then 1 ccm. 1 hour before feeding.

‡ One cubic centimeter standard alkaline extract 1 hour before feeding; or 0.5 ccm. at 18 hours and at 1 hour before feeding.

§ One cubic centimeter standard alkaline extract 1 hour before feeding.

rate, there was now a fair approach to the levels of oxidation and deposition of carbohydrate found in normal animals under the same conditions.

Discussion. Several theories have been advanced to explain the phenomenal hypersensitivity to insulin of hypophysectomized animals, and the "anti-insulin" effects of A.P.E. It has been considered, first, that in the absence of the hypophysis, deficient hepatic glycogenolysis delays recovery from hypoglycemia; secondly, that inadequate gluconeogenesis from non-carbohydrate sources slows recovery; third, that in the absence of a restraining influence on carbohydrate oxidation, the usual effects of insulin on this process are much exaggerated; and lastly, that

A.P.E. may antagonize insulin by interfering with the formation of muscle glycogen from blood sugar.

TABLE 7

The action of insulin and A.P.E. in hypophysectomized rats fed glucose

Terminal analyses made approximately 4 hours after feeding. Respiratory data obtained in interim

	NUMBER OF RATS	GLUCOSE ABSORBED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	OXYGEN CONSUMPTION	R.Q.
		mgm. per 100 grams body wt.	*mgm. per cent*	*mgm. per 100 grams body wt.*	*mgm. per cent*	*ccm. per 100 grams per hour*	
A. A.P.E., 1 ccm. stand. alk. extract given 1 hour before administration of glucose and insulin							
1. No treatment	10	568	79	25	520	107 ±2.7	0.908 ±0.007
2. A.P.E., no insulin	8	457	156	34	502	99 ±3.8	0.812 ±0.008
3. Insulin, 1 unit per kilo, without A.P.E.	10	551	64	17	504	90 ±2.6	0.951 ±0.004
4. Insulin, 1 unit per kilo, with A.P.E.	5	441	93	8	464	106	0.847
5. Insulin, 10 units per kilo, without A.P.E.	7	519	41	2	528	75 ±3.9	0.975 ±0.007
6. Insulin, 10 units per kilo, with A.P.E.	7	495	52*	7*	483	81 ±4.9	0.925 ±0.012
B. A.P.E. given during fasting period, 0.5 ccm. stand. alk. extract given at 24 and 18 hours and at 1 hour before experiment							
1. A.P.E., without insulin	9	531	163*	45	704	110 ±3.5	0.777 ±0.005
2. Insulin, 10 units per kilo, with A.P.E.	6	532	91	39	820	109 ±2.2	0.805 ±0.011
C. Normal rats: Insulin, 10 units per kilo, with A.P.E.	9	735	139	89	872	162 ±2.2	0.787 ±0.004

* Variable.

Early in the investigation of the action of insulin in hypophysectomized animals, it was believed that the chief abnormality lay in the recovery

phase; and indeed comparison of blood sugar curves lent support to this view. In addition it was found that epinephrine appeared to be less active in hypophysectomized animals and that A.P.E. increased this activity. Therefore, in accordance with the prevailing view of the importance of epinephrine action in recovery from insulin hypoglycemia, the theory was widely held that the hypersensitivity to insulin in the absence of the pituitary was due to inability of secreted epinephrine to discharge glycogen stores in these animals. Recently, facts damaging to this view have been published. Zucker and Berg (8), and very recently Swann and Fitzgerald (9), have presented evidence that the removal of the adrenal medulla without serious damage to the adrenal cortex does not greatly increase insulin sensitivity; on this evidence, the rôle of epinephrine in hypophysectomized animals cannot now be considered critical. Russell and Cori (10), and later Heinbecker and Weichselbaum (11), were unable to show that hypophysectomized animals differed from the normal in response to epinephrine when it was administered intravenously or intraperitoneally. Finally, as outlined below, evidence has accumulated showing that A.P.E. need not act in the recovery phase, but can prevent all trace of insulin action from its beginning, and that it may also act in hepatectomized animals.

In the experiments reported here, no further evidence was obtained to indicate failure of glycogenolysis in hypophysectomized animals, or any effect of the A.P.E. on this process. Again it was shown that adrenal-demedullation without great damage to the cortex (in these cases there had been massive regeneration of cortical tissue) did not influence the sensitivity to insulin to nearly the same extent as did hypophysectomy. Further, in all of the fasted rats, normal and hypophysectomized, with and without A.P.E., the liver glycogen levels were extremely low, at most a few milligrams of glycogen being available from this source in any case. Yet the normal and treated animals, compared to the untreated operated rats, remained enormously resistant to insulin, a fact indicating that lack of available glucose was not the cause of hypersensitivity to insulin in the untreated animals. Interference in the Cori cycle in the hypophysectomized rats could not have been a major factor, since adrenal-demedullation, which is assumed to prevent muscle glycogenolysis in hypoglycemia, did not markedly influence the action of insulin.

In the fed rats, the hypersensitivity to insulin of the hypophysectomized rats was not so marked as when they were fasted. It might be considered, therefore, that the hypersensitivity to insulin was principally a reaction phenomenon. However, the fact that hypersensitivity occurred at all when the absorption of glucose was continuing at an undiminished rate, indicates otherwise. Then, as discussed below, another explanation of the observed difference is possible. The failure to deposit hepatic glycogen

in the insulinized fed animals indicates no interference with glycogenolysis in hypoglycemia in these cases. Finally, the A.P.E. was rather more effective as an "anti-insulin agent" in the fed animals, when absorption of glucose was continuing and supplies of glucose from the liver would have been relatively unimportant.

The hypothesis that a deficiency in gluconeogenesis from non-carbohydrate sources was an important factor in the induction of hypersensitivity to insulin in the hypophysectomized animals has never been supported by direct evidence. With the demonstration of a peripheral locus for the contra-insular activity of the A.P.E., this view loses its chief appeal. In these experiments where the A.P.E. was found to affect the hypoglycemic action of the insulin without itself being able to prevent spontaneous hypoglycemia, and where later the phenomena of hypersensitivity to insulin and the "anti-insulin" effect of the A.P.E. were observed in the presence of a plethora of carbohydrate, it is hard to believe that gluconeogenesis can have played much part.

The results of recent experiments on the relationship of the pituitary to carbohydrate metabolism have led to the suggestion that the A.P.E. exerts a restraining influence on the oxidation of carbohydrate (3, 7, 12). It seemed possible, therefore, that the hypersensitivity to insulin of the hypophysectomized animals might be caused by an exaggeration of the action of insulin on carbohydrate oxidation in the absence of the A.P. factors. This view also cannot be said to have received support in the work reported above. In the fasted animals, there is some suggestion of such an effect; but if present, it cannot have been large, and in view of the complications discussed above, the data do not warrant such a conclusion.

In the fed rats, as pointed out, little if any increase in the rate of oxidation of the fed carbohydrate is indicated by the figures. We may consider that in the hypophysectomized rats fed glucose, carbohydrate oxidation was already taking place at very nearly a maximum rate under the circumstances; for as found previously, and as shown in table 3, this rate is approximately the same as that in the normal animals, although the metabolic rate is grossly deficient, and it increases in proportion to the metabolic rate when thyroxin is administered (13, and unpublished data). If this be so, the lack of effect of insulin additional to that already present (and acting) would be explicable. The relative insensitivity of the fed hypophysectomized rats in these experiments would also be in part explained; for if oxidative removal of carbohydrate were limited by the low metabolic rates, then absorption of glucose could keep pace with this process and so maintain the blood sugar levels. Agreeing with this hypothesis is the fact that when not enough glucose had been fed to hypophysectomized rats to maintain absorption throughout the experimental period, the blood sugar levels were low if insulin had been administered

earlier. It is yet possible that experiments under other conditions would demonstrate an exaggeration of insulin action on carbohydrate oxidation.

In a way the most interesting phenomenon encountered in these experiments was the reduction in the oxygen consumption of hypophysectomized rats occasioned by insulin. It is known that small animals, as rats and mice, in a premortal state after prolonged and severe hypoglycemia, may exhibit some decrease in oxygen consumption; but such a condition was not encountered here. Even in the series in which the most marked hypoglycemia was produced (fasted hypophysectomized rats with 0.1 unit of insulin per kilo, with 16 mgm. per cent approximate true blood sugar value), the rats were only rather weak and quiet or occasionally comatose, but never convulsive. This drop in oxygen consumption also occurred in fed rats, when the blood sugar levels were relatively well maintained; in another series of operated animals (adrenal-demedullated, hypophysectomized), in which the blood sugar levels were not as low as those found in normal rats with insulin; and in the hypophysectomized rats treated with smaller amounts of A.P.E., in which there was also but a small drop in blood sugar levels. In all of these series, careful examination in every case revealed no symptoms referable in any way to hypoglycemia. It is true that the blood sugar levels recorded were terminal, whereas the metabolic rates had been determined over the preceding hours. However, since a characteristic of insulin action in hypophysectomized animals is the prolonged hypoglycemia without recovery, it seems most improbable that the blood sugar levels could have been low enough to influence so markedly the oxygen consumption during this time, and then suddenly to have returned to the values recorded. In the normal rats, blood sugar levels as low as 14 mgm. per cent were recorded with but very small changes in oxygen consumption. Therefore, we must believe either that the metabolic regulatory system of the hypophysectomized rat is so defective that but a very small change in blood sugar level is sufficient to cause a marked decrease in metabolic rate; *or that a marked reduction in total oxygen consumption has become a salient characteristic of insulin action in hypophysectomized rats.*

The usual view of insulin action is that it promotes the oxidation of carbohydrate, and it is known that this increase occurs along with an equicaloric reduction in the oxidation of other substances. That is, the insulin may be said to tend to limit oxidation to carbohydrate as the source of energy. Now in the hypophysectomized rats, if carbohydrate oxidation is already occurring at a maximum rate, does the administration of insulin result, not in a further shift toward carbohydrate, but in a reduction in the oxidation of other substances and hence in a reduction in the total oxidation rate? On the other hand, may insulin directly affect the metabolism of other substances? It is certain from the figures presented here that A.P.E. is very active in preventing the effect of insulin

on oxygen uptake, in some cases doing so without greatly affecting the blood sugar levels. If the A.P.E. tends to diminish the rate of oxidation of carbohydrate relative to other substances, as appears from its effects on the R.Q.'s, then its effect on the oxygen consumption in insulinized animals could be produced in this manner.

The metabolic rates of the animals in these experiments were significantly affected by the A.P.E. As pointed out in a previous paper (3), certain A.P. extracts produce an increase of about 10 per cent in fed but not in fasted rats. This effect was here observed in every series of normal fed injected animals, whether insulin was given or not (table 6). In the hypophysectomized rats, metabolic rates were not raised above the level observed in untreated fed animals, but this level was itself much above that prevailing in the operated rats when they are fasted. The effects of insulin on the metabolic rate, on the other hand, were counteracted in both normal and hypophysectomized rats, whether fed or fasted. Immediate effects of A.P.E. on oxygen consumption have been observed previously (14), and have been studied recently by O'Donovan and Collip (15), who indicated that the factor responsible for this activity might be the same as that producing other metabolic effects of the A.P.E.

That insulin promotes the formation of muscle glycogen from blood sugar has long been recognized. Some time ago Marks (4) showed that A.P.E. could antagonize the action of insulin in this respect. More recently, it was found by Himsworth and Scott (2) that in hepatectomized rabbits, A.P.E. could prevent the hypoglycemic action of insulin without at the same time affecting the spontaneous fall in blood sugar in these animals. Probably these two phenomena represent phases of the same action of the A.P.E.—a tendency to inhibit the action of insulin in the deposition of carbohydrate as muscle glycogen. From the experiments reported here, further evidence of this action of the A.P.E. can be adduced. In both normal and hypophysectomized fasted rats, the administration of sufficient A.P.E. prevented the hypoglycemia effects of insulin; but at the same time it did not prevent the spontaneous hypoglycemia of the operated animals or increase the blood sugar levels of the normal rats. In the fed normal rats, antagonism between insulin and A.P.E. in furthering muscle glycogen formation was shown in the prevention of any effect of the insulin in addition to the usual action of A.P.E. on muscle glycogen. The latter activity could not have been on glycogen formation directly, for otherwise it would have added to the effect of the insulin; but probably it was instead the result of the depressing effect on carbohydrate oxidation. From a consideration of these facts, it would appear that some at least of the "anti-insulin" activity of A.P.E. lies in its ability to slow down the deposition of muscle glycogen from blood sugar under the influence of the insulin.

Finally, in all probability a number of factors, or at least different

phases of the action of one or more factors, are concerned in the phenomenon of insulin sensitivity in the absence of the pituitary. In hypophysectomized animals, the action of insulin causing deposition of muscle glycogen may be exaggerated, for the A.P.E. is capable of preventing this deposition in normal rats and probably does so also in hypophysectomized rats. In hypophysectomized rats insulin also exhibits an exaggerated tendency to limit metabolism to carbohydrate as a source of energy; the A.P.E. also opposes this action in both operated and normal rats. The first of these types of action is undoubtedly exhibited by the "anti-insulin" or "glycotropic" factor, which does not appear to be the same as the "diabetogenic" substance (11). Only further study of the separation of the various factors will show the interrelations between these factors and the other metabolic activities of the anterior lobe.

SUMMARY

An investigation has been made of the effects of insulin and of anterior pituitary extract given with insulin on the metabolism of carbohydrate in normal and hypophysectomized rats, in the fasted and fed states. The following are the most important findings:

1. The great hypersensitivity to insulin of fasted hypophysectomized rats was again demonstrated. Under the conditions of these experiments, the difference between similarly effective doses of insulin in the normal and in the hypophysectomized rats was about thirty-fold. Adrenal-demedullation increased the sensitivity to insulin of otherwise normal rats only to a much smaller extent; it also somewhat further increased the sensitivity of animals previously hypophysectomized.

2. *Very small doses of insulin (0.05–0.10 unit per kilo) reduced profoundly the oxygen consumption of fasted hypophysectomized rats, whereas larger doses (up to 3 units per kilo) produced only small changes in the metabolic rates in normal rats.* In a variety of experimental conditions, this reduction in oxygen uptake was not correlated with critical reduction of blood sugar levels.

3. Small doses of insulin in hypophysectomized rats and larger doses in normal rats appeared to raise the R.Q. and to decrease muscle glycogen levels; but these changes were prevented by adrenal-demedullation.

4. In normal rats fed glucose, insulin (1 to 10 units per kilo) caused deposition of muscle glycogen at the expense of the liver glycogen and blood sugar. There was an increase in carbohydrate oxidation only with the largest amount of insulin used. Insulin did not affect the oxygen consumption in these animals.

5. Hypophysectomized rats fed glucose were only moderately more sensitive to the hypoglycemic action of insulin than normal rats similarly treated. The oxygen consumption of these animals, however, was pro-

gressively decreased by increasing doses of insulin. Respiratory quotients were increased parallel to the decreases in oxygen consumption, indicating little if any change in the rate of oxidation of carbohydrate. Blood sugar levels and liver glycogen deposition were diminished by the larger doses of insulin, although these changes could indicate but moderate increase in the utilization of carbohydrate. In view of the marked decrease in the metabolic rates, the insulin would seem to have produced, directly or indirectly, a reduction in the oxidation of non-carbohydrate materials in the hypophysectomized rats.

6. *An alkaline extract of anterior pituitary in appropriate dosage prevented the hypoglycemic action of insulin in both normal and hypophysectomized rats.* The spontaneous fall in blood sugar level occurring in fasted hypophysectomized rats was not affected by this extract. The amount necessary to prevent the action of comparable doses of insulin was many times greater in the hypophysectomized than in normal rats. The action was more efficient when the extract was given during the preceding fast than when given immediately before the insulin.

7. While the administration of either A.P.E. or insulin alone to fed normal rats causes an increase in the deposition of muscle glycogen, antagonism between the substances was demonstrated when the two were given at the same time, and the deposition of muscle glycogen was less than when the insulin was given alone.

8. *The A.P.E. was also able to prevent completely the depression in oxygen consumption produced by the insulin in the hypophysectomized rats.* It also produced a small but consistent increase in oxygen consumption in the fed normal rats, whether insulin was given or not.

9. In reviewing these facts, it was concluded that no evidence was obtained of a marked increase in the rate of oxidation of carbohydrate in hypophysectomized rats given insulin; likewise, evidence could not be adduced that the fault in hypophysectomized animals remains in their reaction to hypoglycemia. It was considered tentatively that the contra-insulin effects of A.P.E. were best explained as produced partly by preventing the withdrawal of glucose from the blood as muscle glycogen and partly by its action in depressing carbohydrate oxidation.

I wish to thank Dr. Herbert M. Evans for his generous support and guidance in this work.

REFERENCES

(1) Russell, J. A. Physiol. Rev. **18:** 1, 1938.
(2) Himsworth, H. P. and D. B. McN. Scott. J. Physiol. **92:** 183, 1938.
(3) Russell, J. A. This Journal **121:** 755, 1938.
(4) Marks, H. P. Proc. Roy. Soc. Med. **29:** 663, 1936; J. Physiol. **87:** 15P, 1936.

(5) Russell, J. A. and L. L. Bennett. Proc. Soc. Exper. Biol. and Med. **34:** 406, 1936.
(6) Russell, J. A. Endocrinology **22:** 80, 1938.
(7) Meyer, H. S., L. V. Wade and C. F. Cori. Proc. Soc. Exper. Biol. and Med. **36:** 346, 1937.
(8) Zucker, T. F. and B. N. Berg. This Journal **119:** 539, 1937.
(9) Swann, H. G. and J. W. Fitzgerald. Endocrinology **22:** 687, 1938.
(10) Russell, J. A. and G. T. Cori. This Journal **119:** 167, 1937.
(11) Heinbecker, P. T. and T. E. Weichselbaum. Proc. Soc. Exper. Biol. and Med. **37:** 527, 1937.
(12) Fisher, R. E., J. A. Russell and C. F. Cori. J. Biol. Chem. **115:** 627, 1936.
(13) Russell, J. A. This Journal **122:** 547, 1938.
(14) Riddle, O., et al. Endocrinology **20:** 1, 1936.
(15) O'Donovan, D. K. and J. B. Collip. This Journal **123:** 157, 1938.
(16) Young, F. G. Biochem. J. **32:** 524, 1938.

THE EFFECT OF CONCENTRATED SERUM ON PLASMA VOLUME AND SERUM PROTEIN CONCENTRATION

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Concentrated sterile blood serum has recently been made available (1, 2). This serum, stored in the form of a dry powder, can readily be dissolved in sterile, distilled water for intravenous use. Because of its rapid solubility, it has been termed "lyophile" serum (1). It has been used in nephrosis in order to increase the concentration of serum proteins for the purpose of drawing edema fluid into the blood stream (3). It has been employed to correct hypoproteinemia and to prevent wound disruption (4). In the presence of a normal serum protein concentration it has been used to draw fluids from tissue spaces in the treatment of hydrocephalus and increased intracranial pressure (5), and experimentally to increase the plasma volume in the treatment of shock (6, 7). The successful use of concentrated serum depends upon its ability to increase the plasma volume by withdrawal of fluid from the tissue spaces. The present experiments were undertaken to measure the changes in plasma volume and serum protein concentration which resulted from the intravenous injection of such a preparation. The changes observed were compared with those which followed the intravenous injection of normal serum, plasma, glucose and saline solutions.

METHODS. Two normal, unanesthetized male dogs were used. Dog 1 was a four year old hound which weighed 25 kgm. Dog 2 was a two year old mongrel which weighed 18 kgm.

Plasma volume determinations were made by the method of Gregersen, Gibson and Stead (8). Dye concentrations were measured with the spectrophotometer set at 6200 milli-micra. Determinations were made in quartz cells 10 mm. in length. Changes in optical density of the plasma reflect changes in plasma volume in accordance with the "indirect method" of plasma volume determinations described by Gibson and Evans (9). Serum proteins were determined in duplicate by the method of Kock and McMeekin (10). Blood for hematocrit determinations, which were made in triplicate by the method of Wintrobe (11), was taken at the same time as that for the dye studies and was mixed under oil with heparin as an anticoagulant. The hematocrit tubes were placed in the centrifuge immediately upon filling and spun for one hour at 1,600 R.P.M.

Since it was impossible to make photometer readings in the presence of lipemia, the animals were given only soda crackers for forty hours before experiments. They had free access to water during this period. Between experiments the dogs were maintained on a diet of beef hearts and milk, and were given additional vitamin B_1. They were given daily exercise except on experimental days.

Before the first blood sample was taken, the animal was allowed a twenty minute rest period. Serial blood samples were taken from the jugular veins. In order to avoid the effects of stasis, a standard routine for sampling was established. Pressure was made at the base of the neck until the needle entered the vein. The pressure was then released and thirty seconds allowed to elapse before withdrawing the blood. A control dye-free sample was taken at the end of the twenty minute rest period.

The dye used in these experiments was obtained through the courtesy of Doctor Gregersen. It was made up in a 1 per cent concentration and sealed in sterile ampoules. The standard of comparison used was a 1:1000 dilution of this 1 per cent dye. Standards made up from four ampoules chosen at random showed a range of optical density of 0.014. Dye injections were made with a 2 cc. Vim syringe calibrated with a metal check arm to deliver 2.000 $\pm$ 0.002 grams of distilled water. All injections were made into the lateral saphenous veins.

The tubes and the syringes used for the collection of the blood were acid cleaned. In order to prevent hemolysis, the tubes and syringes were treated with paraffin oil immediately before the samples were taken.

Following the injection of dye 25 minutes were allowed to elapse before taking a sample of blood, to insure complete mixing of the dye in the blood stream. Six samples at 10 to 15 minute intervals were taken before any fluids were injected intravenously. During the period of observation samples were taken at twenty minute intervals.

Blood samples were allowed to clot under oil at room temperature; the tubes were then centrifuged, the serum pipetted off and recentrifuged.

At the end of the experiment, the amount of blood removed was replaced by its equivalent of citrated blood. The dog was then fed on crackers and given free access to water until the plasma volume was determined the following day by a second injection of dye.

The solutions used in these studies were sterile and sterile apparatus was used for their introduction. The glucose and sodium chloride solutions were injected at the rate of 0.36 to 0.70 cc. per kgm. per minute, the serum at 0.14 to 0.22 cc. per kgm. per minute and the concentrated lyophile serum at 0.08 to 0.13 cc. per kgm. per minute.

The lyophile serum used in these experiments was prepared from normal dogs which had fasted for 36 hours before bleeding. The blood was taken with aseptic precautions through oiled tubing into oiled flasks to prevent

hemolysis. Through the courtesy of Sharpe and Dohme the serum was lyophilized, redissolved, filtered through Berkefeld candles and re-lyophilized. The serum obtained from redissolving this doubly processed serum showed a deviation of +0.014 optical density when compared with a serum sample taken from dog 1.

RESULTS. Figure 1 shows the changes in the optical density of the serum of dog 1 after the injection of 20 mgm. of T-1824 in three experiments, one week apart. The contour of the solid line drawn through these points was used in the subsequent experiments on dog 1 as the standard disappearance curve. From alterations in the shape of this curve, changes in plasma volume induced by the intravenous injection of differ-

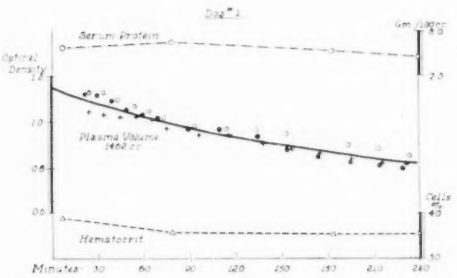

Fig. 1

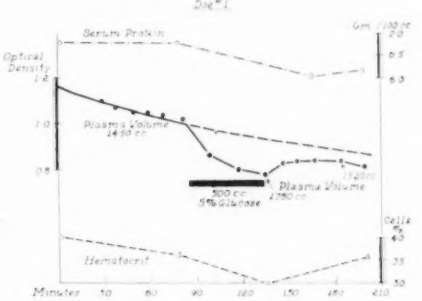

Fig. 2

Fig. 1. Curve of disappearance of dye T-1824 from the blood stream. Dog 1, weight 25 kilo. Twenty milligrams of dye were injected intravenously at zero time on three occasions one week apart. Serum protein concentration and hematocrit values for one experiment are given at top and bottom of chart. In this, as in the following figures, ordinates represent optical density of serum, hematocrit and serum protein concentrations; abscissae, time in minutes.

Fig. 2. Effect of intravenous injection of 500 cc. of 5 per cent glucose on plasma volume, serum protein concentration and hematocrit. Dog 1, 25 kilo.

ent fluids were calculated. A similar curve was drawn for dog 2 from two injections of dye.

The serum protein and hematocrit values varied within narrow limits during the withdrawal of the blood samples, as shown in figure 1.

When 500 cc. of 5 per cent glucose solution in distilled water were injected over a period of 50 minutes the decrease in optical density of the serum, illustrated in figure 2, indicated a prompt increase in plasma volume from 1450 to 1780 cc. This dilution, however, was not maintained. Fifty minutes later the plasma volume had dropped to 1520 cc. The serum protein concentration and hematocrit also indicated a temporary dilution of the plasma. A similar result was observed in dog 2 (table 1; expt. 2).

The effect of the intravenous injection of the physiological salt solution

is shown in figure 3. Although 500 cc. were injected, the maximum increase in plasma volume was less than half that amount. Immediately after the injection, the optical density of the serum increased. The plasma volume then became stabilized at 1550 cc., an increase of 155 cc. over the pre-injection level. The plasma protein concentration and hematocrit indicated similar variations in dilution of plasma. Three additional experiments on the two dogs gave essentially the same results (table 1; expts. 4, 5 and 6).

TABLE 1

Effect of intravenous solutions on plasma volume and serum protein concentration in two normal unanesthetized dogs

EXPERIMENT NO.	DOG NO.	FLUID INJECTED	INITIAL PLASMA VOLUME	CHANGE IN PLASMA VOLUME		PROTEIN INJECTED	SERUM PROTEIN CONCENTRATION		
				Indirect method	Direct 24 hours		Before Injection	Immediately after injection	24 hours after injection
1	1	500 cc. 5% glucose	1450	1520	1438	0	6.84	6.10	6.72
2	2	500 cc. 5% glucose	1102	1102	1150	0	7.14	6.26	6.28
3	1	500 cc. 0.85% NaCl	1395	1550	1740	0	6.84	6.46	6.42
4	1	500 cc. 0.85% NaCl	1290	1490	1675	0	7.06	6.39	6.95
5	1	245 cc. 0.85% NaCl	1552	1680		0			
6	2	500 cc. 0.85% NaCl	1182	1285	1200	0	6.84	5.10	5.72
7	1	500 cc. 5% NaCl	1420	1720	1770	0			
8	1	350 cc. 5% NaCl	1435	1785	1722	0			
9	2	500 cc. 5% NaCl	1162	1200	1212	0			
10	1	380 cc. serum	1490	1830*	1595	21.1	7.66	7.67	7.40
11	1	550 cc. serum	1405	2190*	1680	34.4	7.57	7.73	7.65
12	1	450 cc. serum	1420	1920	1567	33.4	8.66	8.71	7.25
13	1	350 cc. serum	1568	1842	1735‡	23.6	7.50	8.60†	7.25‡
14	1	150 cc. plasma	1505	1740	1510§	7.8	7.13	6.56	7.01§
15	2		1170	1170	1212	0	6.9	6.8	
16	2	40 cc. 4 × conc. serum	992	1135	1268	8.6	7.14	6.96†	7.08
17	2	50 cc. 4 × conc. serum	1102	1440*		10.8			
18	1	50 cc. 4 × conc. serum	1262	1455	1420	11.9	7.0	8.30	7.26
19	1	70 cc. 4 × conc. serum	1505	1735	1575	14.9			
20	1	50 cc. 4 × conc. serum				12.0	6.97	7.26†	6.85

* Urticaria developed during or after injection.
† Dogs and chills.
‡ Six hours after fluid injected intravenously.
§ Forty-eight hours after fluid injected intravenously.

When 5 per cent sodium chloride solution was injected intravenously the dilution of the plasma was more marked, as shown in figure 4. The volume of plasma increased almost precisely by the amount of fluid injected. Dilution was not maintained but the final volume, 1720 cc.,

was still 300 cc. above the initial value. The hematocrit curve followed the dye curve. Three experiments on both dogs gave similar results (table 1; expts. 7, 8 and 9).

The effect of an injection of 380 cc. of normal serum with a protein concentration of 5.55 grams per 100 cc. is shown in figure 5. During the

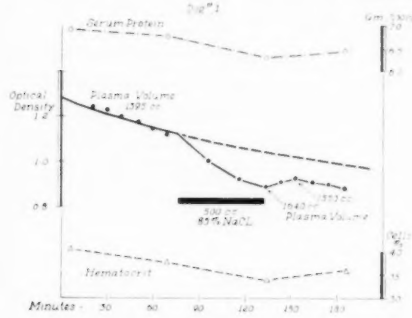

Fig. 3

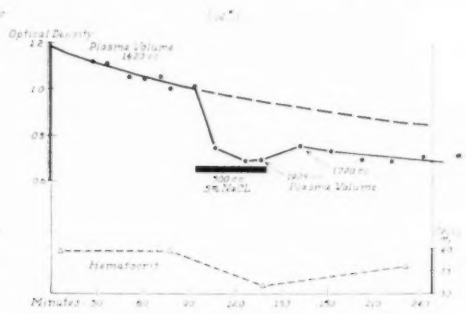

Fig. 4

Fig. 3. Effect of intravenous injection of 500 cc. of 0.85 per cent sodium chloride solution on plasma volume, serum protein concentration and hematocrit. Dog 1, 25 kilo.

Fig. 4. Effect of intravenous injection of 500 cc. of 5 per cent sodium chloride solution on plasma volume and hematocrit. Dog 1, 25 kilo.

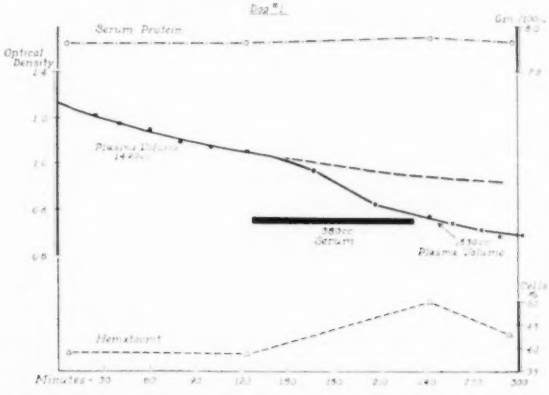

Fig. 5. Effect of intravenous injection of 380 cc. of normal serum with protein concentration of 5.55 grams per 100 cc. on plasma volume, serum protein concentration and hematocrit. Dog 1, 25 kilo.

injection the dye concentration was lowered. The calculated increase in plasma volume, 340 cc., was slightly less than the volume of serum injected, 380 cc. In the succeeding hour, the dye concentration showed no tendency to rise. The serum protein concentration varied only slightly during the

period of observation. A sharp rise in hematocrit occurred at the end of the injection but the value returned toward normal at the end of the experiment. The injection of normal serum or plasma was repeated four times on the same dog with essentially similar results (table 1; expts. 11, 12, 13 and 14).

The results obtained when concentrated "lyophile" serum was injected are shown in figure 6. During the injection of 40 cc. of serum, concentrated from an initial quantity of 160 cc., the dilution of the dye indicated an increase in plasma volume (143 cc.), approximately four times the quantity of fluid injected. The serum protein and hematocrit changed only slightly. The dilution of the dye was maintained for the remainder of the experiment. This observation was confirmed in four additional experiments (table 1; expts. 17, 18, 19 and 20).

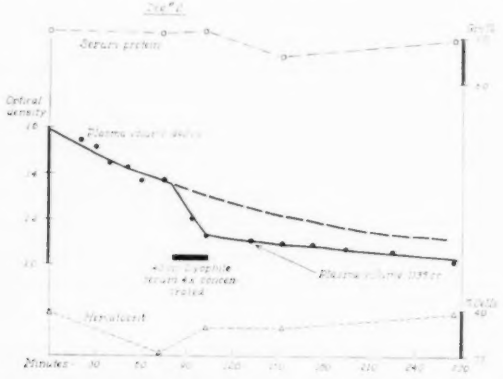

Fig. 6. Effect of intravenous injection of 40 cc. of lyophile serum with protein concentration of 21.6 grams per 100 cc. on plasma volume, serum protein concentration and hematocrit. Dog 2, 18 kilo.

Discussion. The dye used in these experiments, T-1824, is not diffusible and is not excreted by the kidneys, but is removed from the blood stream by the reticulo-endothelial system (9). It disappears from the blood stream at 5 to 8 per cent per hour (8). In determinations of the plasma volume from the disappearance slope over short periods of time (20–60 min.) the assumption has been made by other investigators (8, 9) that the dye leaves the blood stream at a constant rate. Figure 1 shows, however, that the rate of disappearance varies with the concentration of the dye. The error introduced by the assumption of a linear disappearance is but slight when the time of observation is small. In these experiments, however, the time of observation was extended to four hours. We were unable to develop a mathematical formula for the curve of disappearance.

It therefore became necessary to determine for each dog, over the experimental period, the contour of the disappearance curve with the same initial concentration of dye. The curve for the disappearance of the dye from the blood stream during the experimental period shown in figure 1 is the mean of 41 observations of optical density. The maximum deviation from the mean of a single determination is less than ± 5 per cent. Since in all the experiments several observations were used in the calculations of the changes in plasma volume by the "indirect" method, it is unlikely that the error exceeded 5 per cent. With this curve as a base line, changes induced by the injection of different solutions were studied.

The absorption spectra of many vital dyes in plasma is altered by variations in the concentration of sodium chloride and protein. Gregersen and Gibson (12) have shown that the absorption spectra of the dye T-1824 is not affected by fluctuations in the salt or protein content of circulating blood.

The temporary increase in plasma volume which follows the intravenous injection of glucose or sodium chloride solutions has been calculated by many investigators from change in the hematocrit, red blood count, hemoglobin or serum protein concentration (13, 14, 15, 16, 17 and 18). Smith (14) was the first to make repeated determinations of the plasma volume with the dye method, and showed that such an increase occurred. Miller and Poindexter, however, in similar experiments (16) were unable to obtain reliable results with this method due to the presence of residual dye in the serum. Gregersen (19) has analyzed the deficiencies of colorimetric methods in relation to plasma volume determinations and has pointed out the advantages of using spectrophotometric readings with the blue dye T-1824. The results illustrated in figures 2, 3 and 4 are in accordance with other observations in which spectrophotometric measurements of this dye were made during the injection of glucose and sodium chloride solutions (9, 18).

When normal serum was injected (fig. 5) a sustained increase in plasma volume was observed by the dye method. This result is in agreement with the measurements of Blalock, Beard and Thuss (15) who used alterations in hematocrit and serum protein concentration in anesthetized dogs to calculate changes in plasma volume. Robertson (17) on the contrary, in anesthetized cats, found that the serum had no more lasting effect on plasma volume than sodium chloride solutions. He injected 44 cc. per kgm. at the rate of 4 cc. per kgm. per minute. The volume of serum injected was twice as large as that used in our experiments and the rate of injection twenty times as fast. In our unanesthetized dogs, when an attempt was made to inject serum more rapidly (0.7 cc. per kgm. per minute) signs of over-distention of the vascular bed appeared. The veins became distended, the dog went into collapse with low blood pressure,

pallor of the mucous membrane and a weak, rapid pulse. Under such conditions the venous blood became quite dark. The rise in hematocrit illustrated in figure 5 was associated with a mild reaction of this nature. Since Robertson (17) used changes in hemoglobin concentration from which to calculate alterations in the plasma volume, and since it is recognized that stasis causes concentration of the blood (20), it is possible that the physiological reactions to overloading of the circulation affected the results. Boycott (21) has called attention to the pathological condition found in anesthetized rabbits after massive injection of gelatin or serum. Since the physiological conditions surrounding the experiments of Robertson were not ideal we cannot agree with his statement that "the mechanism for the maintenance of fluid equilibrium cannot altogether be explained on purely physical grounds" (17).

The increase in plasma volume, measured by the "indirect" dye method, which resulted from the injection of serum in our experiments was approximately equal to the volume of serum injected. On three occasions (table 1; expts. 10, 11 and 17) the dogs developed reactions from the serum which resulted in urticaria. The wheals were colored blue by the dye in the plasma. The measured increase in plasma volume was greater on two of these occasions than the theoretical increase, probably because some of the dye was lost from the blood stream into the wheals.

The dogs also occasionally suffered chills. Stasis occurred and resulted in hemoconcentration (table 1; expts. 13, 16 and 20). Barbour and Hamilton (20) have called attention to the concentration of the blood associated with chilling of the body. Whenever the serum protein concentration was increased by the injection of normal or concentrated serum, a rise in the hematocrit reading was also found which indicated that hemoconcentration had occurred.

The plasma volume and serum protein concentration were measured again 24 hours after the injection. A small increase was observed when serum was injected but no greater than that found after the injection of glucose or sodium chloride solutions.

When serum which had been concentrated to one-fourth its initial volume was injected, immediate dilution took place so that the plasma volume was raised by approximately four times the quantity of fluid injected. The concentration of serum protein was unaffected except when hemoconcentration occurred. Since the dogs did not have access to water during the interval, the fluid for dilution of the serum protein and for increase in plasma volume must have come from the extravascular fluid reservoirs. Peters and Van Slyke (22) have stated: "The plasma protein concentration may be affected either by changing the total amount of circulating protein in the body or by changing the blood plasma volume so that the proteins are diluted or concentrated." The experiments on the injection

of concentrated serum demonstrate the fact that the serum protein concentration is maintained constant by expansion of the plasma volume. The colloidal osmotic pressure of the serum obligates the plasma volume in accordance with Starling's original concept of the function of the serum proteins.

CONCLUSIONS

1. Intravenous injection of concentrated serum into normal, unanesthetized dogs produces an increase in plasma volume by drawing fluid from the extravascular reservoirs.

2. Intravenous injection of normal serum increases the plasma volume without increase in serum protein concentration.

3. Intravenous injection of glucose and sodium chloride solutions produces a rise in plasma volume with dilution of the serum proteins and hematocrits but the dilution of the blood is not maintained.

REFERENCES

(1) Flosdorf, E. W. and S. Mudd. J. Immunology **29:** 389, 1935.
(2) Flosdorf, E. W. and S. Mudd. J. Immunology **34:** 469, 1938.
(3) Aldrich, C. A., J. Stokes, Jr., W. P. Killingsworth and A. C. McGuiness. J. A. M. A. **111:** 129, 1938.
(4) Thompson, W. D., I. S. Ravdin, J. E. Rhoads and I. L. Frank. Arch. Surg. **36:** 509, 1938.
(5) Hughes, J., S. Mudd and E. A. Strecker. Trans. Am. Neurol. Assn. **62:** 118, 1936.
(6) Bond, D. D. and D. G. Wright. Ann. Surg. **107:** 500, 1938.
(7) Mahoney, E. B. Ann. Surg. **108:** 178, 1938.
(8) Gregersen, M. I., J. G. Gibson, 2nd and E. A. Stead. This Journal **113:** 54, 1935.
(9) Gibson, J. G., 2nd and W. A. Evans, Jr. J. Clin. Investigation **16:** 301, 1937.
(10) Koch, F. C. and T. L. McMeekin. J. Am. Chem. Soc. **46:** 2066, 1924.
(11) Wintrobe, M. M. Am. J. Med. Sc. **185:** 58, 1933.
(12) Gregersen, M. I. and J. G. Gibson, 2nd. This Journal **120:** 494, 1937.
(13) Smith, A. H. and L. B. Mendel. This Journal **53:** 323, 1920.
(14) Smith, H. P. Bull. Johns Hopkins Hosp. **37:** 177, 1925.
(15) Blalock, A., J. W. Beard and C. Thuss. J. Clin. Investigation **11:** 267, 1932.
(16) Miller, J. R. and C. A. Poindexter. J. Lab. and Clin. Med. **18:** 287, 1933.
(17) Robertson, J. D. Brit. J. Exper. Path. **19:** 30, 1938.
(18) Gilligan, D. R., M. D. Altschule and M. C. Volk. J. Clin. Investigation **17:** 7, 1938.
(19) Gregersen, M. I. J. Lab. and Clin. Med. **23:** 423, 1938.
(20) Barbour, H. G. and W. F. Hamilton. This Journal **73:** 315, 1925.
(21) Boycott, A. E. J. Path. and Bact. **18:** 498, 1913.
(22) Peters, J. P. and D. D. Van Slyke. Quantitative clinical chemistry. Williams & Wilkins, 1931, vol. 1, p. 662.

THE INFLUENCE OF HYDROGENATION AND OXIDATION OF FATS UPON THEIR RATE OF ABSORPTION[1,2]

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The work of Holmes and Deuel (1921) on the digestibility of hydrogenated fats has suggested that as the melting point of a fat increases above 46°C. its total digestibility decreases. In Holmes' experiments 92 per cent or more of the hydrogenated fats melting below 46° were digested. Of a hydrogenated peanut oil melting at 52.4° and a hydrogenated corn oil melting at 50.0°, only 79 and 88.5 per cent respectively were digested. The total digestibility was found to decrease at a faster rate with fats which melted above 46° than with those which melted below this temperature.

It was of interest to determine if the rate of absorption of fats was affected as well. Accordingly a vegetable oil was hydrogenated to iodine numbers ranging from 44.7 to 108.3 thereby giving melting points ranging from below body temperature to well above it (10° to 49°C.). The resultant preparations were fed by stomach tube to adult rats. Oxidized fats were fed in a similar manner for a determination of the effect of rancidity upon the rate of absorption.

EXPERIMENTAL. The rats were fasted 48 hours and then given 1.5 cc. of fat by stomach tube. After a four-hour absorption period analyses were made of the residues in the stomach and small intestine and cecum as already described (Irwin, Steenbock, and Templin, 1936).

The effect of hydrogenation upon the rate of fat absorption. Preliminary experiments were carried out with a sample of hydrogenated butter oil. The butter oil was prepared as follows: 240 grams of butter fat were dissolved in 1500 cc. of absolute alcohol at 45°C., cooled to room temperature, 23.5°C., and the solid fats filtered off. The filtrate was allowed to stand

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in a refrigerator over night at 1°C. The solid fats were again removed and the solvent distilled off in a partial vacuum with nitrogen at 40–45°C. Any alcohol that remained was removed by heating the fat one hour in a vacuum oven at 92°C. and allowing it to stand over night in a vacuum desiccator. The yield of oil was 33 grams. Part of this was hydrogenated.[3] Both the original oil and the hydrogenated product were fed to rats and the percentage of fat absorbed in 4 hours determined. Seventy-one per cent of the butter oil was found to be absorbed in 4 hours, whereas of the hydrogenated product only 58 per cent was absorbed. The two

TABLE 1

The percentage of fats of different melting points absorbed in four hours by adult male rats

GROUP	KIND OF FAT FED	FAT ABSORBED	MELTING POINT OF FAT	IODINE VALUE OF FAT	NUMBER OF RATS
		per cent		*Wijs*	
1	Butter oil	71 ±1.2	3–5	40.1	11
2	Hydrogenated butter oil	58 ±2.4	42.5	3.1	12
3	Hydrogenated vegetable oil #1 A	47 ±1.3	10–15	108.3	11
4	Hydrogenated vegetable oil #2 A	49 ±1.4	25.1	103.1	9
5	Hydrogenated vegetable oil #3 A	48 ±2.0	30.0	95.8	11
6	Hydrogenated vegetable oil #4 A	49 ±1.9	33.0	91.4	10
7	Hydrogenated vegetable oil #6 A	49 ±1.0	36.2	81.1	10
8	Hydrogenated vegetable oil #8 A	48 ±1.2	37.8	71.8	12
9	Hydrogenated vegetable oil #9 A	45 ±1.1	40.0	66.9	11
10	Hydrogenated vegetable oil #10 A	38 ±1.6	42.0	62.2	10
11	Hydrogenated vegetable oil H49 #4	26 ±2.0	49.0	44.7	8

SIGNIFICANCE OF DIFFERENCES	MEAN DIFFERENCE	STANDARD DEVIATION OF MEAN DIFFERENCE	MD / σMD
Difference between groups (1) and (2)	13	2.2	5.9
Difference between groups (8) and (10)	10	2.9	3.4
Difference between groups (9) and (10)	7	2.8	2.5

fats melted[4] at 3–5°C. and 42.5°C. and had iodine numbers of 40.1 and 3.1 respectively (table 1).

The effect of hydrogenation upon fat absorption was studied further

[3] All of the experimental hydrogenated fats used in these experiments were hydrogenated in the laboratories of Lever Brothers Company, Edgewater, New Jersey.

[4] All melting points were determined by the Wiley method as outlined in *Methods of Analysis of the Association of Official Agricultural Chemists*, 3rd edition, Washington, 1930, p. 317. However a slight variation in procedure was followed. In the Wiley method the temperature reading is taken at the point where the suspended disc becomes a sphere. In our experiments this sphere was allowed to become transparent and the reading taken the instant the sphere became clear. This reading seemed to be the one most successfully duplicated.

with cottonseed oil. This was hydrogenated in part[5] and at intervals during the process samples were removed, bleached, and deodorized. Each sample was fed to a group of rats and the percentage of fat absorbed determined. The data (table 1) show clearly that as the melting point increased up to body temperature, 37 to 38°C. (groups 3 to 8), the rate of absorption

TABLE 2

The percentage of fat absorbed in four hours by adult rats fed 1.5 cc. of melted fats

KIND OF FAT FED (5)	FAT ABSORBED	MELTING POINT OF FAT	IODINE VALUE OF FAT	NUMBER OF RATS PER GROUP
	per cent	°C.	*Hanus*	
Hydrogenated vegetable shortening B	53 ±2.3	45.0	67.5	10
Hydrogenated vegetable shortening A	54 ±1.6	43.7	66.5	11
Hydrogenated animal shortening C	44 ±0.9	48.8	58.5	9
Hydrogenated animal shortening F	39 ±1.2	46.4	54.6	11

TABLE 3

The percentage of fat absorbed in four hours by adult rats fed oxidized fat

GROUP	SAMPLE NUMBER	NUMBER OF HOURS AERATED	IODINE VALUE (HANUS)	PEROXIDE NUMBER OF FAT	NUMBER OF RATS	PER CENT OF FAT ABSORBED	MELTING POINT °C.
1	Lot 57, #16	0	72.0	0.5	13	58 ±2.4	45.6
2	Lot 57, #10	2	71.9	12.9	11	56 ±1.9	45.8
3	Lot 57, #11	4	71.6	27.0	10	46 ±1.4	44.8
4	Lot 57, #12	6	70.8	57.6	11	44 ±1.2	45.6
5	Lot 57, #13	8	69.5	80.1	11	41 ±2.0	45.6
6	Lot 57, #14	10	67.4	107.6	11	38 ±1.2	45.3
7	Lot 57, #15	12	66.4	127.7	10	37 ±2.2	46.9

SIGNIFICANCE OF DIFFERENCES	MEAN DIFFERENCE	STANDARD DEVIATION OF MEAN DIFFERENCE	$\frac{MD}{\sigma MD}$
Difference between groups (1) and (2)	2	4.5	0.4
Difference between groups (1) and (3)	12	4.1	3.0
Difference between groups (1) and (4)	14	4.0	3.5
Difference between groups (1) and (5)	17	4.6	3.7
Difference between groups (1) and (6)	20	3.9	5.1
Difference between groups (1) and (7)	21	4.7	4.5

remained constant. However, when the melting point rose above body temperature, the rate of absorption decreased (groups 9, 10, and 11). The differences between groups 8 and 10 and also 9 and 10 are not to be taken as due to chance but as true differences on a statistical basis (table 1). A

[5] The fats tested were available commercially. Hydrogenated vegetable shortening A and B were the same fats reported on in a previous paper (Steenbock, Irwin, and Weber, 1936). Hydrogenated animals fats C and F were products commonly appearing on the market.

higher melting sample of the same hydrogenation was not available, but of a similarly hydrogenated vegetable oil melting at 49°C., only 26 per cent was absorbed in four hours (group 11, table 1).

It is evident from these data that variations in the melting point below body temperature had little effect upon the rate of absorption, but as the melting point exceeded body temperature the rate of absorption decreased.

Four commercially hydrogenated fats, two of vegetable origin and two animal fats, were tested for their rate of absorption at four hours in the same manner as before. The results of these tests are presented in table 2. Again the highest melting fats were absorbed less rapidly.

The effect of the presence of oxidation products. Samples of hydrogenated cottonseed oil were oxidized by aerating 75 grams of the fat with 10 liters of air per hour at a temperature of 130°C. for 2, 4, 6, 8, 10, or 12 hours.[6] The rate of absorption of these oxidized fats was determined as described. The data (table 3) show that as the oxidation of the fat proceeded, its rate of absorption diminished. The mean difference in the percentage of fat absorbed from sample to sample is, in many cases, not statistically significant. For instance, the difference between the percentages of fat absorbed by group 1 and group 2, 2 per cent, might be due to chance, but there is a real difference in the absorption shown by groups 1 and 3, or between groups 1 and 4, 5, 6, or 7. Since the differences in melting point were not sufficient to account for the change in absorption the effect can be ascribed to oxidation.

The percentage of fat absorbed during a 12-hour absorption period was determined for two of the oxidized fats, lot 57, no. 14 and lot 57, no. 15. These samples showed absorptions of 90 and 92 per cent respectively. Apparently, these oxidized fats are not quite as completely absorbed in 12 hours as unoxidized fats are, the latter being 97 to 99 per cent absorbed in the same length of time (Steenbock, Irwin and Weber, 1936).

SUMMARY

1. The rate of absorption of fat was found to decrease as the melting point increased above body temperature. Variations in melting point below body temperature had no effect upon the rate of absorption.

2. The rate of absorption of a hydrogenated fat was found to vary inversely with the degree of oxidation.

REFERENCES

Holmes, A. D. and H. J. Deuel. This Journal **54**: 479, 1921.
Irwin, M. H., H. Steenbock and V. M. Templin. J. Nutrition **12**: 85, 1936.
Steenbock, H., M. H. Irwin and J. Weber. J. Nutrition **12**: 103, 1936.

[6] The oxidations were conducted and the iodine values and peroxide numbers determined in the laboratories of Lever Brothers Company, Edgewater, New Jersey.

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